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(54) Title: 65499 AND 58875, NOVEL SEVEN-TRANSMEMBRANE RECEPTORS AND USES THEREFOR

65499 AND 58875, NOVEL SEVEN-TRANSMEMBRANE RECEPTORS AND USES THEREFOR

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195 196 197 198 199 200 201 202 203 204 205 206 207 208 209 210 211 212 213 214 215 216 217 218 219 220 221 222 223 224 225 226 227 228 229 230 231 232 233 234 235 236 237 238 239 240 241 242 243 244 245 246 247 248 249 250 251 252 253 254 255 256 257 258 259 260 261 262 263 264 265 266 267 268 269 270 271 272 273 274 275 276 277 278 279 280 281 282 283 284 285 286 287 288 289 290 291 292 293 294 295 296 297 298 299 300 301 302 303 304 305 306 307 308 309 310 311 312 313 314 315 316 317 318 319 320 321 322 323 324 325 326 327 328 329 330 331 332 333 334 335 336 337 338 339 340 341 342 343 344 345 346 347 348 349 350 351 352 353 354 355 356 357 358 359 360 361 362 363 364 365 366 367 368 369 370 371 372 373 374 375 376 377 378 379 380 381 382 383 384 385 386 387 388 389 390 391 392 393 394 395 396 397 398 399 400 401 402 403 404 405 406 407 408 409 410 411 412 413 414 415 416 417 418 419 420 421 422 423 424 425 426 427 428 429 430 431 432 433 434 435 436 437 438 439 440 441 442 443 444 445 446 447 448 449 450 451 452 453 454 455 456 457 458 459 460 461 462 463 464 465 466 467 468 469 470 471 472 473 474 475 476 477 478 479 480 481 482 483 484 485 486 487 488 489 490 491 492 493 494 495 496 497 498 499 500 501 502 503 504 505 506 507 508 509 510 511 512 513 514 515 516 517 518 519 520 521 522 523 524 525 526 527 528 529 530 531 532 533 534 535 536 537 538 539 540 541 542 543 544 545 546 547 548 549 550 551 552 553 554 555 556 557 558 559 560 561 562 563 564 565 566 567 568 569 570 571 572 573 574 575 576 577 578 579 580 581 582 583 584 585 586 587 588 589 590 591 592 593 594 595 596 597 598 599 600 601 602 603 604 605 606 607 608 609 610 611 612 613 614 615 616 617 618 619 620 621 622 623 624 625 626 627 628 629 630 631 632 633 634 635 636 637 638 639 640 641 642 643 644 645 646 647 648 649 650 651 652 653 654 655 656 657 658 659 660 661 662 663 664 665 666 667 668 669 670 671 672 673 674 675 676 677 678 679 680 681 682 683 684 685 686 687 688 689 690 691 692 693 694 695 696 697 698 699 700 701 702 703 704 705 706 707 708 709 710 711 712 713 714 715 716 717 718 719 720 721 722 723 724 725 726 727 728 729 730 731 732 733 734 735 736 737 738 739 740 741 742 743 744 745 746 747 748 749 750 751 752 753 754 755 756 757 758 759 760 761 762 763 764 765 766 767 768 769 770 771 772 773 774 775 776 777 778 779 780 781 782 783 784 785 786 787 788 789 790 791 792 793 794 795 796 797 798 799 800 801 802 803 804 805 806 807 808 809 810 811 812 813 814 815 816 817 818 819 820 821 822 823 824 825 826 827 828 829 830 831 832 833 834 835 836 837 838 839 840 841 842 843 844 845 846 847 848 849 850 851 852 853 854 855 856 857 858 859 860 861 862 863 864 865 866 867 868 869 870 871 872 873 874 875 876 877 878 879 880 881 882 883 884 885 886 887 888 889 890 891 892 893 894 895 896 897 898 899 900 901 902 903 904 905 906 907 908 909 910 911 912 913 914 915 916 917 918 919 920 921 922 923 924 925 926 927 928 929 930 931 932 933 934 935 936 937 938 939 940 941 942 943 944 945 946 947 948 949 950 951 952 953 954 955 956 957 958 959 960 961 962 963 964 965 966 967 968 969 970 971 972 973 974 975 976 977 978 979 980 981 982 983 984 985 986 987 988 989 990 991 992 993 994 995 996 997 998 999 1000

A

65499 AND 58875, NOVEL SEVEN-TRANSMEMBRANE RECEPTORS AND USES THEREFOR

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195 196 197 198 199 200 201 202 203 204 205 206 207 208 209 210 211 212 213 214 215 216 217 218 219 220 221 222 223 224 225 226 227 228 229 230 231 232 233 234 235 236 237 238 239 240 241 242 243 244 245 246 247 248 249 250 251 252 253 254 255 256 257 258 259 260 261 262 263 264 265 266 267 268 269 270 271 272 273 274 275 276 277 278 279 280 281 282 283 284 285 286 287 288 289 290 291 292 293 294 295 296 297 298 299 300 301 302 303 304 305 306 307 308 309 310 311 312 313 314 315 316 317 318 319 320 321 322 323 324 325 326 327 328 329 330 331 332 333 334 335 336 337 338 339 340 341 342 343 344 345 346 347 348 349 350 351 352 353 354 355 356 357 358 359 360 361 362 363 364 365 366 367 368 369 370 371 372 373 374 375 376 377 378 379 380 381 382 383 384 385 386 387 388 389 390 391 392 393 394 395 396 397 398 399 400 401 402 403 404 405 406 407 408 409 410 411 412 413 414 415 416 417 418 419 420 421 422 423 424 425 426 427 428 429 430 431 432 433 434 435 436 437 438 439 440 441 442 443 444 445 446 447 448 449 450 451 452 453 454 455 456 457 458 459 460 461 462 463 464 465 466 467 468 469 470 471 472 473 474 475 476 477 478 479 480 481 482 483 484 485 486 487 488 489 490 491 492 493 494 495 496 497 498 499 500 501 502 503 504 505 506 507 508 509 510 511 512 513 514 515 516 517 518 519 520 521 522 523 524 525 526 527 528 529 530 531 532 533 534 535 536 537 538 539 540 541 542 543 544 545 546 547 548 549 550 551 552 553 554 555 556 557 558 559 560 561 562 563 564 565 566 567 568 569 570 571 572 573 574 575 576 577 578 579 580 581 582 583 584 585 586 587 588 589 590 591 592 593 594 595 596 597 598 599 600 601 602 603 604 605 606 607 608 609 610 611 612 613 614 615 616 617 618 619 620 621 622 623 624 625 626 627 628 629 630 631 632 633 634 635 636 637 638 639 640 641 642 643 644 645 646 647 648 649 650 651 652 653 654 655 656 657 658 659 660 661 662 663 664 665 666 667 668 669 670 671 672 673 674 675 676 677 678 679 680 681 682 683 684 685 686 687 688 689 690 691 692 693 694 695 696 697 698 699 700 701 702 703 704 705 706 707 708 709 710 711 712 713 714 715 716 717 718 719 720 721 722 723 724 725 726 727 728 729 730 731 732 733 734 735 736 737 738 739 740 741 742 743 744 745 746 747 748 749 750 751 752 753 754 755 756 757 758 759 760 761 762 763 764 765 766 767 768 769 770 771 772 773 774 775 776 777 778 779 780 781 782 783 784 785 786 787 788 789 790 791 792 793 794 795 796 797 798 799 800 801 802 803 804 805 806 807 808 809 810 811 812 813 814 815 816 817 818 819 820 821 822 823 824 825 826 827 828 829 830 831 832 833 834 835 836 837 838 839 840 841 842 843 844 845 846 847 848 849 850 851 852 853 854 855 856 857 858 859 860 861 862 863 864 865 866 867 868 869 870 871 872 873 874 875 876 877 878 879 880 881 882 883 884 885 886 887 888 889 890 891 892 893 894 895 896 897 898 899 900 901 902 903 904 905 906 907 908 909 910 911 912 913 914 915 916 917 918 919 920 921 922 923 924 925 926 927 928 929 930 931 932 933 934 935 936 937 938 939 940 941 942 943 944 945 946 947 948 949 950 951 952 953 954 955 956 957 958 959 960 961 962 963 964 965 966 967 968 969 970 971 972 973 974 975 976 977 978 979 980 981 982 983 984 985 986 987 988 989 990 991 992 993 994 995 996 997 998 999 1000

B

(57) Abstract: The invention provides isolated nucleic acids molecules, designated 65499 and 58875 nucleic acid molecules, which encode novel seven transmembrane domain (7TM) receptors, e.g., G-protein coupled receptor family members. The invention also provides antisense nucleic acid molecules, recombinant expression vectors containing 65499 or 58875 nucleic acid molecules, host cells into which the expression vectors have been introduced, and nonhuman transgenic animals in which a 65499 or 58875 gene has been introduced or disrupted. The invention still further provides isolated 65499 or 58875 proteins, fusion proteins, antigenic peptides and anti-65499 or 58875 antibodies. Diagnostic methods utilizing of the invention are also provided.

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One type of receptor family is the seven transmembrane domain (7TM) receptor family. This receptor family is characterized structurally by the presence of seven hydrophobic, membrane-spanning regions, as well as an intracellular domain and an extracellular ligand binding domain. Members of the 7TM receptor family typically are G-protein coupled receptors (GPCRs). G-protein coupled receptors are proteins that mediate signal transduction of a diverse number of ligands through heterotrimeric G proteins (*see, e.g., Strader (1994) Annu. Rev. Biochem.* 63:101-132). GPCRs are a component of many modular cell signaling systems involving, *e.g.,* G proteins, intracellular enzymes and channels. Upon ligand binding to a GPCR, intracellular signal molecules, *e.g.,* G proteins, can be activated or turned off. These GPCR-coupled G proteins can modulate the activity of different intracellular effector molecules, *e.g.,* enzymes and ion channels (*see, e.g., Gutkind (1998) J. Biol. Chem.* 273: 1839-1842; Selbie (1998) *Trends Pharmacol. Sci.* 19:87-93).

The intracellular domain(s) of GPCRs bind G proteins, which represent a family of heterotrimeric proteins comprising of α , β and γ subunits. G proteins typically bind guanine nucleotides. Following ligand binding to the GPCR, a conformational change is transmitted from the extracellular GPCR ligand binding domain to the intracellular domain-bound G protein. This causes the G protein α -subunit to exchange a bound GDP molecule for a GTP molecule and to dissociate from the $\beta\gamma$ -subunits. The GTP-bound form of the α -subunit typically functions as an effector-modulating moiety, leading to the production of second messengers, such as, *e.g.*, cyclic AMP (*e.g.*, by activation of adenylate cyclase), diacylglycerol or inositol phosphates.

GPCRs are of critical importance in cell signaling systems, including the endocrine system, the central nervous system and peripheral physiological processes. GPCRs are the receptors of different families of neuropeptides, and neuropeptides are involved in nociception. The GPCR genes and gene-products can also be causative agents of disease (see, e.g., Spiegel (1993) *J. Clin. Invest.* 92:1119-1125; McKusick (1993) *J. Med. Genet.*

30:1-26). Given the important biological roles and properties of GPCRs, there exists a need for the identification and characterization of novel GPCR genes and proteins as well as for the discovery of binding agents (*e.g.*, ligands) and modulators of these nucleic acids and polypeptides for use in regulating a variety of normal and/or pathological cellular
5 processes.

Summary of the Invention

The present invention is based, in part, on the discovery of novel seven transmembrane domain (7TM) receptors, with similarities to the rhodopsin family of 7TM
10 receptors, and nucleic acids encoding these receptors, referred to herein collectively as "7TMRs," or by the individual clone names "65499" and "58875."

The nucleotide sequence of a cDNA encoding 65499 is shown in SEQ ID NO:1, and the amino acid sequence of a 65499 polypeptide is shown in SEQ ID NO:2. The nucleotide sequence of a cDNA encoding 58875 is shown in SEQ ID NO:3, and the amino acid sequence
15 of a 58875 polypeptide is shown in SEQ ID NO:4. In addition, the nucleotide sequence of the coding region of a 65499 polypeptide is depicted in SEQ ID NO:5 and the nucleotide sequence of the coding region of a 58875 polypeptide is depicted in SEQ ID NO:6.

Accordingly, in one aspect, the invention features a nucleic acid molecule which encodes a 65499 or 58875 protein or polypeptide, *e.g.*, a biologically active portion of the
20 65499 or 58875 protein. In a preferred embodiment the isolated nucleic acid molecule encodes a polypeptide having the amino acid sequence of SEQ ID NO:2 or 4. In other embodiments, the invention provides isolated 65499 or 58875 nucleic acid molecules having the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO: 5 or SEQ ID NO: 6, or the sequence of the DNA insert of one of the plasmids deposited with
25 ATCC Accession Numbers _____. In still other embodiments, the invention provides nucleic acid molecules that are substantially identical (*e.g.*, naturally occurring allelic variants) to the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO: 5 or SEQ ID NO: 6; or the sequence of the DNA insert of one of the plasmids deposited with ATCC Accession Numbers _____. In other embodiments, the invention provides a nucleic
30 acid molecule which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO: 5 or SEQ ID NO: 6; or the sequence of the DNA insert of one of the plasmids

deposited with ATCC Accession Numbers ____, wherein the nucleic acid encodes a full length 65499 or 58875 protein or an active fragment thereof.

In a related aspect, the invention further provides nucleic acid constructs which include a 65499 or 58875 nucleic acid molecule described herein. In certain embodiments, the nucleic acid molecules of the invention are operatively linked to native or heterologous regulatory sequences. Also included, are vectors and host cells containing the 65499 or 58875 nucleic acid molecules of the invention, *e.g.*, vectors and host cells suitable for producing 65499 or 58875 nucleic acid molecules and polypeptides.

In another related aspect, the invention provides nucleic acid fragments suitable as primers or hybridization probes for the detection of 65499 or 58875-encoding nucleic acids.

In still another related aspect, isolated nucleic acid molecules that are antisense to a 65499 or 58875 encoding nucleic acid molecule are provided.

In another aspect, the invention features, 65499 or 58875 polypeptides, and biologically active or antigenic fragments thereof that are useful, *e.g.*, as reagents or targets in assays applicable to treatment and diagnosis of 65499- or 58875-mediated or related disorders. In another embodiment, the invention provides 65499 or 58875 polypeptides having a 65499 or 58875 activity. Preferred polypeptides are 65499 or 58875 proteins including at least one, two, three, four, five, six or seven transmembrane domains, and, preferably, having a 65499 or 58875 activity, *e.g.*, a 65499 or 58875 activity as described herein. Preferred polypeptides are 65499 or 58875 proteins including at least one seven transmembrane domain.

In other embodiments, the invention provides 65499 or 58875 polypeptides, *e.g.*, a 65499 or 58875 polypeptide having the amino acid sequence shown in SEQ ID NO:2 or SEQ ID NO: 4; an amino acid sequence encoded by the cDNA insert of one of the plasmids deposited with ATCC Accession Numbers ____; an amino acid sequence that is substantially identical to the amino acid sequence shown in SEQ ID NO:2 or SEQ ID NO: 4; or an amino acid sequence encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO: 5, or SEQ ID NO: 6; or the sequence of the DNA insert of one of the plasmids deposited with ATCC Accession Numbers ____, wherein the nucleic acid encodes a full length 65499 or 58875 protein or an active fragment thereof.

In a related aspect, the invention further provides nucleic acid constructs which include a 65499 or 58875 nucleic acid molecule described herein.

In a related aspect, the invention provides 65499 or 58875 polypeptides or fragments operatively linked to non-65499 or 58875 polypeptides to form fusion proteins.

5 In another aspect, the invention features antibodies and antigen-binding fragments thereof, that react with, or more preferably specifically bind 65499 or 58875 polypeptides.

In another aspect, the invention provides methods of screening for compounds that modulate the expression or activity of the 65499 or 58875 polypeptides or nucleic acids.

In still another aspect, the invention provides a process for modulating 65499 or
10 58875 polypeptide or nucleic acid expression or activity, *e.g.* using the screened compounds described herein. In certain embodiments, the methods involve treatment of conditions related to aberrant activity or expression of the 65499 or 58875 polypeptides or nucleic acids, such as conditions involving aberrant or deficient transmission of an extracellular signal into a cell; aberrant or deficient mobilization of an intracellular
15 molecule that participates in a signal transduction pathway; and/or aberrant or deficient modulation of function, survival, morphology, proliferation and/or differentiation of cells of tissues in which 65499 or 58875 molecules are expressed.

The invention also provides assays for determining the activity of or the presence or absence of 65499 or 58875 polypeptides or nucleic acid molecules in a biological sample,
20 including for disease diagnosis.

In a further aspect, the invention provides assays for determining the presence or absence of a genetic alteration in a 65499 or 58875 polypeptide or nucleic acid molecule, including for disease diagnosis.

In another aspect, the invention features a two dimensional array having a plurality
25 of addresses, each address of the plurality being positionally distinguishable from each other address of the plurality, and each address of the plurality having a unique capture probe, *e.g.*, a nucleic acid or peptide sequence. At least one address of the plurality has a capture probe that recognizes a 65499 or 58875 molecule. In one embodiment, the capture probe is a nucleic acid, *e.g.*, a probe complementary to a 65499 or 58875 nucleic acid
30 sequence. In another embodiment, the capture probe is a polypeptide, *e.g.*, an antibody specific for 65499 or 58875 polypeptides. Also featured is a method of analyzing a sample by contacting the sample to the aforementioned array and detecting binding of the sample to the array.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

Brief Description of the Drawings

5 *Figures 1A-B* depict a cDNA sequence (SEQ ID NO:1) and predicted amino acid sequence (SEQ ID NO:2) of human 65499 receptor. The location of the methionine-initiated open reading frame of human 65499 (without the 5' and 3' untranslated regions) is also indicated in the Figure (SEQ ID NO:5)

10 *Figure 2* depicts a hydropathy plot of human 65499. Relatively hydrophobic residues are shown above the dashed horizontal line, and relatively hydrophilic residues are below the dashed horizontal line. The cysteine residues (cys) and N-glycosylation sites (Ngly) are indicated by short vertical lines just below the hydropathy trace. The location of the transmembrane domains and the extracellular and intracellular portions are also indicated. The numbers corresponding to the amino acid sequence of human 65499 are indicated. Polypeptides of the invention include fragments which include: all or part of a
15 hydrophobic sequence, e.g., a sequence above the dashed line, e.g., the sequence from about amino acids 70 to 90, from about 195 to 215, and from about 400 to 420 of SEQ ID NO:2; all or part of a hydrophilic sequence, e.g., a sequence below the dashed line, e.g., the sequence from about amino acid 250 to 270, from about 320 to 340, and from about 475 to
20 490 of SEQ ID NO:2; or a sequence which includes a Cys, or a glycosylation site.

25 *Figure 3* depicts an alignment of the 7 transmembrane receptor family domain of human 65499 with a consensus amino acid sequence derived from a hidden Markov model (HMM) from PFAM. The upper sequences are the consensus amino acid sequence (SEQ ID NO:7), while the lower amino acid sequences correspond to amino acids 48 to 454 of SEQ ID NO:2.

30 *Figure 4* depicts a BLAST alignment of human 65499 with a consensus amino acid sequence derived from a ProDomain No. PD000009, "Receptor coupled G-protein transmembrane glycoprotein phosphorylation lipoprotein palmitate family multigene" (Release 2001.1; <http://www.toulouse.inra.fr/prodom.html>). The lower sequence is amino acid residues 4 to 128 of the 131 amino acid consensus sequence (SEQ ID NO:8), while the upper amino acid sequence corresponds to the "Receptor coupled G-protein transmembrane glycoprotein phosphorylation lipoprotein palmitate family multigene" domain of human 65499, amino acid residues 61 to 170 of SEQ ID NO:2.

Figures 5a-b depict a BLAST alignment of human 65499 with a consensus amino acid sequence derived from a ProDomain No. PD180341, "RE2 receptor coupled G-protein" (Release 2001.1; <http://www.toulouse.inra.fr/prodom.html>). The lower sequences are amino acid residues 99 to 172 and 5 to 50 of the 241 amino acid consensus sequence (SEQ ID NOs:9 and 10), while the upper amino acid sequence corresponds to the "RE2 receptor coupled G-protein" domains of human 65499, amino acid residues 394 to 468 and 175 to 220 of SEQ ID NO:2.

Figures 6a-b depict a BLAST alignment of human 65499 with a consensus amino acid sequence derived from a ProDomain No. PD155019, "Receptor type hypocretin EG:22E5.10 EG:22E5.11 transmembrane coupled orexin G-protein" (Release 2001.1; <http://www.toulouse.inra.fr/prodom.html>). The lower sequences are amino acid residues 2 to 61 and 95 to 167 of the 227 amino acid consensus sequence (SEQ ID NOs:11 and 12), while the upper amino acid sequence corresponds to the "Receptor type hypocretin EG:22E5.10 EG:22E5.11 transmembrane coupled orexin G-protein" domains of human 65499, amino acid residues 170 to 228 and 396 to 463 of SEQ ID NO:2.

Figure 7 depicts a BLAST alignment of human 65499 with a consensus amino acid sequence derived from a ProDomain No. PD047622, "GTP-binding rhodopsin" (Release 2001.1; <http://www.toulouse.inra.fr/prodom.html>). The lower sequence is amino acid residues 85 to 284 of the 322 amino acid consensus sequence (SEQ ID NO:13), while the upper amino acid sequence corresponds to the "GTP-binding rhodopsin" domain of human 65499, amino acid residues 28 to 212 of SEQ ID NO:2.

Figure 8 depicts a cDNA sequence (SEQ ID NO:3) and predicted amino acid sequence (SEQ ID NO:4) of human 58875 receptor. The location of the methionine-initiated open reading frame of human 58875 (without the 5' and 3' untranslated regions) is also indicated in the Figure (SEQ ID NO:6)

Figure 9 depicts a hydropathy plot of human 58875. Relatively hydrophobic residues are shown above the dashed horizontal line, and relatively hydrophilic residues are below the dashed horizontal line. The cysteine residues (cys) and N-glycosylation sites (Ngly) are indicated by short vertical lines just below the hydropathy trace. The location of the transmembrane domains and the extracellular and intracellular portions are also indicated. The numbers corresponding to the amino acid sequence of human 58875 are indicated. Polypeptides of the invention include fragments which include: all or part of a hydrophobic sequence, e.g., a sequence above the dashed line, e.g., the sequence from

about amino acids 30 to 50, from about 200 to 220, and from about 285 to 295 of SEQ ID NO:4; all or part of a hydrophilic sequence, e.g., a sequence below the dashed line, e.g., the sequence from about amino acid 140 to 150, from about 225 to 235, and from about 315 to 330 of SEQ ID NO:4; or a sequence which includes a Cys, or a glycosylation site.

5 *Figure 10* depicts an alignment of the 7 transmembrane receptor family domain (rhodopsin family) of human 58875 with a consensus amino acid sequence derived from a hidden Markov model (HMM) from PFAM. The upper sequences are the consensus amino acid sequence (SEQ ID NO:14), while the lower amino acid sequences correspond to amino acids 51 to 306 of SEQ ID NO:4.

10 *Figure 11* depicts a BLAST alignment of human 58875 with a consensus amino acid sequence derived from a ProDomain No. PD000009, "Receptor coupled G-protein transmembrane glycoprotein phosphorylation lipoprotein palmitate family multigene" (Release 2001.1; <http://www.toulouse.inra.fr/prodom.html>). The lower sequence is amino acid residues 6 to 120 of the 131 amino acid consensus sequence (SEQ ID NO:15), while
15 the upper amino acid sequence corresponds to the "Receptor coupled G-protein transmembrane glycoprotein phosphorylation lipoprotein palmitate family multigene" domain of human 58875, amino acid residues 65 to 175 of SEQ ID NO:4.

Figure 12 depicts a BLAST alignment of human 58875 with a consensus amino acid sequence derived from a ProDomain No. PD061380, "Receptor SLC-1 protein-coupled G-protein transmembrane GPR24" (Release 2001.1;
20 <http://www.toulouse.inra.fr/prodom.html>). The lower sequence is amino acid residues 1 to 65 of the 79 amino acid consensus sequence (SEQ ID NO:16), while the upper amino acid sequence corresponds to the "Receptor SLC-1 protein-coupled G-protein transmembrane GPR24" domain of human 58875, amino acid residues 270 to 334 of SEQ ID NO:4.

25 *Figure 13* depicts a BLAST alignment of human 58875 with a consensus amino acid sequence derived from a ProDomain No. PD310793, "Receptor orphan GPR26 protein-coupled" (Release 2001.1; <http://www.toulouse.inra.fr/prodom.html>). The lower sequence is amino acid residues 95 to 196 of the 205 amino acid consensus sequence (SEQ ID NO:17), while the upper amino acid sequence corresponds to the "Receptor orphan
30 GPR26 protein-coupled" domain of human 58875, amino acid residues 235 to 339 of SEQ ID NO:4.

Figure 14 depicts a BLAST alignment of human 58875 with a consensus amino acid sequence derived from a ProDomain No. PD191540, "Receptor H10E21.2 protein-

coupled ZC84.4 G-protein glycoprotein transmembrane" (Release 2001.1; <http://www.toulouse.inra.fr/prodom.html>). The lower sequence is amino acid residues 4 to 166 of the 378 amino acid consensus sequence (SEQ ID NO:18), while the upper amino acid sequence corresponds to the "Receptor H10E21.2 protein-coupled ZC84.4 G-protein glycoprotein transmembrane" domain of human 58875, amino acid residues 26 to 189 of SEQ ID NO:4.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

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Detailed Description of the Invention

Human 65499

The human 65499 nucleotide sequence (Figures 1a-b; SEQ ID NO:1), which is approximately 1704 nucleotides long including untranslated regions, contains a predicted methionine-initiated coding sequence of about 1527 nucleotides, including the termination codon (nucleotides indicated as coding of SEQ ID NO:1 in Fig. 1; SEQ ID NO:3). The coding sequence encodes a 508 amino acid protein (SEQ ID NO:2).

This mature protein form is approximately 508 amino acid residues in length (from about amino acid 1 to amino acid 508 of SEQ ID NO:2). The human 65499 protein contains the following structural features:

20 a predicted seven transmembrane (7TM) domain (PFAM Accession Number PF00001) located at about amino acids 48 to 454 of SEQ ID NO:2;

a predicted seven transmembrane domain (predicted by MEMSAT, Jones *et al.* (1994) *Biochemistry* 33:3038-3049) at about amino acids 32-56, 73-97, 106-127, 149-170, 194-218, 400-424, and 434-456 of SEQ ID NO:2;

25 three predicted N-glycosylation sites (Prosites PS00001) from about amino acids 7-10, 13-16, and 361-364 of SEQ ID NO:2;

two predicted cAMP- and cGMP-dependent protein kinase phosphorylation sites (Prosites PS00004) from about amino acids 230-233 and 380-383 of SEQ ID NO:2;

eight predicted protein kinase C phosphorylation sites (Prosites PS00005) from about amino acids 8-10, 66-68, 144-146, 283-285, 324-326, 331-333, 379-381, and 460-462 of SEQ ID NO:2;

30 five predicted casein kinase II phosphorylation sites (Prosites PS00006) from about amino acids 8-11, 296-299, 325-328, 363-366, and 483-486 of SEQ ID NO:2;

six predicted N-myristoylation sites (Prosite PS00008) from about amino acids 31-36, 187-192, 282-287, 292-297, 316-321, and 489-494 of SEQ ID NO:2;

one predicted prokaryotic membrane lipoprotein lipid attachment site (Prosite PS00013) from about amino acids 201-211 of SEQ ID NO:2; and

5 one predicted G-protein coupled receptors signature site (Prosite PS00237) from about amino acids 117-133 of SEQ ID NO:2.

For general information regarding PFAM identifiers, PS prefix and PF prefix domain identification numbers, refer to Sonnhammer *et al.* (1997) *Protein* 28:405-420 and <http://www.psc.edu/general/software/packages/pfam/pfam.html>.

10 A plasmid containing the nucleotide sequence encoding human 65499 was deposited with American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, on _____ and assigned Accession Number _____. This deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. This
15 deposit was made merely as a convenience for those of skill in the art and is not an admission that a deposit is required under 35 U.S.C. §112.

Human 58875

The human 58875 nucleotide sequence (Figure 8; SEQ ID NO:3), which is
20 approximately 1278 nucleotides long including untranslated regions, contains a predicted methionine-initiated coding sequence of about 1023 nucleotides including the termination codon (nucleotides indicated as coding of SEQ ID NO:3 in Fig.8; SEQ ID NO:6). The coding sequence encodes a 340 amino acid protein (SEQ ID NO:4).

This mature protein form is approximately 340 amino acid residues in length (from
25 about amino acid 1 to amino acid 340 of SEQ ID NO:4). The human 58875 protein contains the following structural features:

a predicted seven transmembrane (7TM) domain (PFAM Accession Number PF00001) located at about amino acids 51 to 306 of SEQ ID NO:4;

a predicted seven transmembrane domain (predicted by MEMSAT, Jones *et al.*
30 (1994) *Biochemistry* 33:3038-3049) at about amino acids 36-60, 70-90, 99-115, 153-171, 199-223, 252-270, and 288-309 of SEQ ID NO:4;

two predicted N-glycosylation sites (Prosite PS00001) from about amino acids 10-13 and 17-20 of SEQ ID NO:4;

two predicted cAMP- and cGMP-dependent protein kinase phosphorylation sites (Prosite PS00004) from about amino acids 63-66 and 322-325 of SEQ ID NO:4;

four predicted protein kinase C phosphorylation sites (Prosite PS00005) from about amino acids 62-64, 150-152, 325-327, and 335-337 of SEQ ID NO:4;

5 six predicted casein kinase II phosphorylation sites (Prosite PS00006) from about amino acids 11-14, 30-33, 66-69, 110-113, 189-192, and 325-328 of SEQ ID NO:4;

five predicted N-myristoylation sites (Prosite PS00008) from about amino acids 42-47, 48-53, 102-107, 156-161, and 180-185 of SEQ ID NO:4;

one predicted G-protein coupled receptors signature site (Prosite PS00237) from
10 about amino acids 119-135 of SEQ ID NO:4; and

one predicted microbodies C-terminal targeting signal (Prosite PS00342) from about amino acids 338-340 of SEQ ID NO:4.

For general information regarding PFAM identifiers, PS prefix and PF prefix domain identification numbers, refer to Sonnhammer *et al.* (1997) *Protein* 28:405-420 and
15 <http://www.psc.edu/general/software/packages/pfam/pfam.html>.

A plasmid containing the nucleotide sequence encoding human 58875 was deposited with American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, on _____ and assigned Accession Number _____. This deposit will be maintained under the terms of the Budapest Treaty on the International
20 Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. This deposit was made merely as a convenience for those of skill in the art and is not an admission that a deposit is required under 35 U.S.C. §112.

The 65499 and 58876 receptors of the present invention contains a significant number of structural characteristics in common with members of the 7TM receptor family,
25 including the G-protein coupled receptor family. The term "family" when referring to the protein and nucleic acid molecules of the invention means two or more proteins or nucleic acid molecules having a common structural domain or motif and having sufficient amino acid or nucleotide sequence homology as defined herein. Such family members can be naturally or non-naturally occurring and can be from either the same or different species.
30 For example, a family can contain a first protein of human origin as well as other distinct proteins of human origin, or alternatively, can contain homologues of non-human origin, *e.g.*, rat or mouse proteins. Members of a family can also have common functional characteristics.

As used herein, the term “seven transmembrane domain receptor” or “7TM” or “7TMR” refers to a family of proteins that preferably comprise an N-terminal extracellular domain, seven transmembrane domains (also referred to as membrane-spanning domains), three extracellular domains (also referred to as extracellular loops), three cytoplasmic domains (also referred to as cytoplasmic loops), and a C-terminal cytoplasmic domain (also referred to as a cytoplasmic tail).

As used herein, the term “G protein-coupled receptor” or “GPCR” refers to a family of proteins that preferably comprise an N-terminal extracellular domain, seven transmembrane domains (also referred to as membrane-spanning domains), three extracellular domains (also referred to as extracellular loops), three cytoplasmic domains (also referred to as cytoplasmic loops), and a C-terminal cytoplasmic domain (also referred to as a cytoplasmic tail). Members of the GPCR family also share certain conserved amino acid residues, some of which have been determined to be critical to receptor function and/or G protein signaling. For example, GPCRs usually contain the following features including a conserved asparagine residue in the first transmembrane domain. An alignment of the transmembrane domains of 44 representative GPCRs can be found at <http://mgdsk1.nid1.nih.gov:8000/extended.html>.

Based on structural similarities, members of the 7TMR family have been classified into various subfamilies, including: Subfamily I which comprises receptors typified by rhodopsin and the beta2-adrenergic receptor and currently contains over 200 unique members (reviewed by Dohlman *et al.* (1991) *Annu. Rev. Biochem.* 60:653-688); Subfamily II, which includes the parathyroid hormone/calcitonin/secretin receptor family (Juppner *et al.* (1991) *Science* 254:1024-1026; Lin *et al.* (1991) *Science* 254:1022-1024); Subfamily III, which includes the metabotropic glutamate receptor family in mammals, such as the GABA receptors (Nakanishi *et al.* (1992) *Science* 258: 597-603); Subfamily IV, which includes the cAMP receptor family that is known to mediate the chemotaxis and development of *D. discoideum* (Klein *et al.* (1988) *Science* 241:1467-1472); and Subfamily V, which includes the fungal mating pheromone receptors such as STE2 (reviewed by Kurjan I *et al.* (1992) *Annu. Rev. Biochem.* 61:1097-1129). Within each family, distinct, highly conserved motifs have been identified. These motifs have been suggested to be critical for the structural integrity of the receptor, as well as for coupling to G proteins.

Based on the results from the HMM analysis (HMMER Version 2.1.1), the 65499 and 58875 polypeptides appears to belong to the rhodopsin subfamily of 7TMRs (family 1).

As used herein, a "65499 or 58875 activity", "biological activity of 65499 or 58875" or "functional activity of 65499 or 58875", refers to an activity exerted by a 65499 or 58875 protein, polypeptide or nucleic acid molecule on *e.g.*, a 65499- or 58875-responsive cell or on a 65499 or 58875 substrate, *e.g.*, a protein substrate, as determined *in vivo* or *in vitro*. In one embodiment, a 65499 or 58875 activity is a direct activity, such as an association with a 65499 or 58875 target molecule. A "target molecule" or "binding partner" is a molecule with which a 65499 or 58875 protein binds or interacts in nature. A 65499 or 58875 activity can also be an indirect activity, *e.g.*, a cellular signaling activity mediated by interaction of the 65499 or 58875 receptor with a 65499 or 58875 ligand.

10 The 65499 or 58875 molecules of the present invention are predicted to have similar biological activities as 7TM receptor family members, *e.g.*, G-protein coupled receptor family members. For example, the 65499 or 58875 proteins of the present invention can have one or more of the following activities: (1) regulating, sensing and/or transmitting an extracellular signal into a cell, (for example, a heart cell, a bone cell (*e.g.*,
15 an osteoclast or an osteoblast), a hematopoietic cell, a neural cell); (2) interacting with (*e.g.*, binding to) an extracellular signal or a cell surface receptor; (3) mobilizing an intracellular molecule that participates in a signal transduction pathway (*e.g.*, adenylate cyclase or phosphatidylinositol 4,5-bisphosphate (PIP₂), inositol 1,4,5-triphosphate (IP₃)); (4) regulating polarization of the plasma membrane; (5) controlling production or secretion of
20 molecules; (6) altering the structure of a cellular component; (7) modulating cell proliferation, *e.g.*, synthesis of DNA; and (8) modulating cell migration, cell differentiation; and cell survival. Thus, the 65499 or 58875 molecules can act as novel diagnostic targets and therapeutic agents for controlling G-protein coupled receptor-related disorders. Other activities, as described below, include the ability to modulate function, survival,
25 morphology, proliferation and/or differentiation of cells of tissues in which 65499 or 58875 molecules are expressed.

Many available therapeutic drugs in use today target GPCRs, as they mediate vital physiological responses, including vasodilation, heart rate, bronchodilation, endocrine secretion, and gut peristalsis. See, *eg.*, Lefkowitz et al., *Ann. Rev. Biochem.* 52:159 (1983).
30 For example, ligands to beta adrenergic receptors are used in the treatment of anaphylaxis, shock, hypertension, hypotension, asthma and other conditions. Additionally, spontaneous activation of GPCRs occurs, where a GPCR cellular response is generated in the absence of

a ligand. Increased spontaneous activity can be decreased by antagonists of the GPCR (a process known as inverse agonism); such methods are therapeutically important where diseases cause an increase in spontaneous GPCR activity. Thus, modulation of the activity of the 65499 or 58875 molecules of the invention may be beneficial in modulating a variety of physiological responses, such as vasodilation, heart rate, bronchodilation, endocrine secretion or gut peristalsis. Moreover, downmodulation of the 65499 or 58875 molecules of the invention can be beneficial in conditions characterized by increased spontaneous activity of 65499 or 58875. Furthermore, the 65499 and 58875 molecules of the invention are members of the rhodopsin family of 7TM receptors. Rhodopsin is a visual pigment which is a sensor for recognizing optical information and a membrane protein widely distributed in vertebrate and invertebrate species. Rhodopsin is useful as a material for photosensor or optical information recognition elements. Thus, the 65499 and 58875 molecules of the invention also may prove useful as materials for photosensor or optical information recognition elements.

The response mediated by a 65499 or 58875 receptor protein depends on the type of cell. For example, in some cells, binding of a ligand to the receptor protein may stimulate an activity such as release of compounds, gating of a channel, cellular adhesion, migration, differentiation, etc., through phosphatidylinositol or cyclic AMP metabolism and turnover while in other cells, the binding of the ligand will produce a different result. Regardless of the cellular activity/response modulated by the receptor protein, GPCRs of the 7TM family interacts with G proteins to produce one or more secondary signals, in a variety of intracellular signal transduction pathways, *e.g.*, through phosphatidylinositol or cyclic AMP metabolism and turnover, in a cell. As used herein, a "signaling transduction pathway" refers to the modulation (*e.g.*, stimulation or inhibition) of a cellular function/activity upon the binding of a ligand to the GPCR (65499 or 58875 protein). Examples of such functions include mobilization of intracellular molecules that participate in a signal transduction pathway, *e.g.*, phosphatidylinositol 4,5-bisphosphate (PIP₂), inositol 1,4,5-triphosphate (IP₃) and adenylate cyclase.

As used herein, "phosphatidylinositol turnover and metabolism" refers to the molecules involved in the turnover and metabolism of phosphatidylinositol 4,5-bisphosphate (PIP₂) as well as to the activities of these molecules. PIP₂ is a phospholipid found in the cytosolic leaflet of the plasma membrane. Binding of ligand to the receptor activates, in some cells, the plasma-membrane enzyme phospholipase C that in turn can hydrolyze PIP₂ to

produce 1,2-diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP₃). Once formed IP₃ can diffuse to the endoplasmic reticulum surface where it can bind an IP₃ receptor, *e.g.*, a calcium channel protein containing an IP₃ binding site. IP₃ binding can induce opening of the channel, allowing calcium ions to be released into the cytoplasm. IP₃ can also be phosphorylated by a specific kinase to form inositol 1,3,4,5-tetraphosphate (IP₄), a molecule which can cause calcium entry into the cytoplasm from the extracellular medium. IP₃ and IP₄ can subsequently be hydrolyzed very rapidly to the inactive products inositol 1,4-bisphosphate (IP₂) and inositol 1,3,4-triphosphate, respectively. These inactive products can be recycled by the cell to synthesize PIP₂. The other second messenger produced by the hydrolysis of PIP₂, namely 1,2-diacylglycerol (DAG), remains in the cell membrane where it can serve to activate the enzyme protein kinase C. Protein kinase C is usually found soluble in the cytoplasm of the cell, but upon an increase in the intracellular calcium concentration, this enzyme can move to the plasma membrane where it can be activated by DAG. The activation of protein kinase C in different cells results in various cellular responses such as the phosphorylation of glycogen synthase, or the phosphorylation of various transcription factors, *e.g.*, NF- κ B. The language "phosphatidylinositol activity", as used herein, refers to an activity of PIP₂ or one of its metabolites.

Another signaling pathway in which the receptor may participate is the cAMP turnover pathway. As used herein, "cyclic AMP turnover and metabolism" refers to the molecules involved in the turnover and metabolism of cyclic AMP (cAMP) as well as to the activities of these molecules. Cyclic AMP is a second messenger produced in response to ligand-induced stimulation of certain G protein coupled receptors. In the cAMP signaling pathway, binding of a ligand to a GPCR can lead to the activation of the enzyme adenylyl cyclase, which catalyzes the synthesis of cAMP. The newly synthesized cAMP can in turn activate a cAMP-dependent protein kinase. This activated kinase can phosphorylate a voltage-gated potassium channel protein, or an associated protein, and lead to the inability of the potassium channel to open during an action potential. The inability of the potassium channel to open results in a decrease in the outward flow of potassium, which normally repolarizes the membrane of a neuron, leading to prolonged membrane depolarization.

To identify the presence of a 7 transmembrane receptor profile in a 65499 or 58875 receptor, and make the determination that a polypeptide or protein of interest has a particular profile, the amino acid sequence of the protein can be searched against the Pfam database of HMMs (*e.g.*, the Pfam database, release 2.1) using the default parameters

(http://www.sanger.ac.uk/Software/Pfam/HMM_search). For example, the hmmsf program, which is available as part of the HMMER package of search programs, is a family specific default program for PF00001 and a score of 15 is the default threshold score for determining a hit. Alternatively, the threshold score for determining a hit can be lowered (e.g., to 8 bits). A description of the Pfam database can be found in Sonhammer *et al.* (1997) *Proteins* 28:405-420 and a detailed description of HMMs can be found, for example, in Gribskov *et al.* (1990) *Meth. Enzymol.* 183:146-159; Gribskov *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:4355-4358; Krogh *et al.* (1994) *J. Mol. Biol.* 235:1501-1531; and Stultz *et al.* (1993) *Protein Sci.* 2:305-314, the contents of which are incorporated herein by reference. Alternatively, the seven transmembrane domain can be predicted based on stretches of hydrophobic amino acids forming α -helices (SOUSI server). For example, using a SOUSI server, a 7 TM receptor profile was identified in the amino acid sequence of SEQ ID NO:2 (e.g., amino acids 32 to 456 of SEQ ID NO:2) and in the amino acid sequence of SEQ ID NO: 4 (e.g., amino acids 36 to 309 of SEQ ID NO:4). Accordingly, 65499 or 58875 proteins having at least 50-60% homology, preferably about 60-70%, more preferably about 70-80%, or about 80-90% homology with the 7 transmembrane receptor profile of human 65499 or 58875 are within the scope of the invention.

In one embodiment, a 65499 or 58875 protein includes at least one "7 transmembrane receptor" domain or regions homologous with a "7 transmembrane receptor" domain. As used herein, the term "7 transmembrane receptor" domain includes an amino acid sequence having at least about 10-350 amino acid residues in length and having a bit score for the alignment of the sequence to the 7tm_1 family Hidden Markov Model (HMM) of at least 8. Preferably, a "7 transmembrane receptor family" domain includes at least about 50-350 amino acid residues, more preferably about 100-350 amino acid residues, or at least about 200-350 amino acids in length and having a bit score for the alignment of the sequence to the "7 transmembrane receptor family" domain (HMM) of at least 16, 50, 100, 150 or greater. The "7 transmembrane receptor family" domain (HMM) has been assigned the PFAM Accession PF00001 (http://pfam.wustl.edu/cgi-bin/getdesc?name=7tm_1). An alignment of the "7 transmembrane receptor family" domain (amino acids 48 to 454 of SEQ ID NO:2 and 51 to 306 of SEQ ID NO:4) of human 65499 and 58875 with a consensus amino acid sequence derived from a hidden Markov model is depicted in Figures 3 and 10.

Preferably, the 7 transmembrane receptor family domain includes the following amino acid consensus sequence having Prosite signatures as PS00237 or PS50262, or sequences homologous thereto: [GSTALIVMFYWC]-[GSTANCPDE]-{EDPKRH}-x(2)-[LIVMNQGA]-x(2)-[LIVMFT]-[GSTANC]-[LIVMFYWSTAC]-[DENH]-R-[FYWCSH]-x(2)-[LIVM] (SEQ ID NO:19). In addition, the 7 transmembrane receptor family domain may include the following amino acid consensus sequence having Prosite signature PS00238, or sequences homologous thereto: [LIVMFWAC]-[PGAC]-x(3)-[SAC]-K-[STALIMR]-[GSACPNV]-[STACP]-x(2)-[DENF]-[AP]-x(2)-[IY] (SEQ ID NO:20). In the above conserved motifs, and other motifs described herein, the standard IUPAC one-letter code for the amino acids is used. Each element in the pattern is separated by a dash (-); square brackets ([]) indicate the particular residues that are accepted at that position; x indicates that any residue is accepted at that position; and numbers in parentheses (()) indicate the number of residues represented by the accompanying amino acid.

In a preferred embodiment, 65499 or 58875 polypeptide or protein has a “7 transmembrane receptor domain” or a region which includes at least about x amino acid residues and has at least about 60%, 70%, 80%, 90%, 95%, 99%, or 100% homology with a “7 transmembrane receptor domain,” e.g., the 7 transmembrane receptor domain of human 65499 (e.g. amino acid residues 48-454 of SEQ ID NO:2) or human 58875 (e.g. amino acid residues 51-306 of SEQ ID NO:4).

For further identification of domains in a 65499 or 58875 protein sequence, and make the determination that a polypeptide or protein of interest has a particular profile, the amino acid sequence of the protein can be searched against a database of domains, e.g., the ProDom database (Corpet et al. (1999), Nucl. Acids Res. 27:263-267). The ProDom protein domain database consists of an automatic compilation of homologous domains. Current versions of ProDom are built using recursive PSI-BLAST searches (Altschul SF et al. (1997) *Nucleic Acids Res.* 25:3389-3402; Gouzy et al. (1999) 23:333-340) of the SWISS-PROT 38 and TREMBL protein databases. The database automatically generates a consensus sequence for each domain.

A BLAST search was performed against the HMM database resulting in the identification of regions homologous to ProDom family PD000009 (“Receptor coupled G-protein transmembrane glycoprotein phosphorylation lipoprotein palmitate family multigene” SEQ ID NO:8, ProDomain Release 2001.1; <http://www.toulouse.inra.fr/prodom.html>). An alignment of the “Receptor coupled G-

protein transmembrane glycoprotein phosphorylation lipoprotein palmitate family multigene" domain (amino acids 61-170 of SEQ ID NO:2) of human 65499 with consensus amino acid sequences (SEQ ID NO:8) derived from a hidden Markov model is depicted in Figure 4. The consensus sequence for SEQ ID NO:8 is 35% identical over amino acids 61 to 170 of SEQ ID NO:2 as shown in Figure 4.

A BLAST search was performed against the HMM database resulting in the identification of regions homologous to ProDom family PD180341("RE2 receptor coupled G-protein" SEQ ID NOs:9 and 10, ProDomain Release 2001.1; <http://www.toulouse.inra.fr/prodom.html>). An alignment of the "RE2 receptor coupled G-protein" domain (amino acids 394-468 and 175-220 of SEQ ID NO:2) of human 65499 with consensus amino acid sequences (SEQ ID NOs:9 and 10) derived from a hidden Markov model is depicted in Figures 5a-b. The consensus sequence for SEQ ID NO:9 is 32% identical over amino acids 394 to 468 and the consensus sequence for SEQ ID NO:10 is 34% identical over amino acids 175 to 220 of SEQ ID NO:2 as shown in Figures 5a-b.

A BLAST search was performed against the HMM database resulting in the identification of regions homologous to ProDom family PD155019("Receptor type hypocretin EG:22E5.10 EG:22E5.11 transmembrane coupled orexin G-protein" SEQ ID NOs:11 and 12, ProDomain Release 2001.1; <http://www.toulouse.inra.fr/prodom.html>). An alignment of the "Receptor type hypocretin EG:22E5.10 EG:22E5.11 transmembrane coupled orexin G-protein" domain (amino acids 170-228 and 396-463 of SEQ ID NO:2) of human 65499 with consensus amino acid sequences (SEQ ID NOs:11 and 12) derived from a hidden Markov model is depicted in Figures 6a-b. The consensus sequence for SEQ ID NO:11 is 33% identical over amino acids 170 to 228 and the consensus sequence for SEQ ID NO:12 is 20% identical over amino acids 396 to 463 of SEQ ID NO:2 as shown in Figures 6a-b.

A BLAST search was performed against the HMM database resulting in the identification of regions homologous to ProDom family PD047622("GTP-binding rhodopsin" SEQ ID NO:13, ProDomain Release 2001.1; <http://www.toulouse.inra.fr/prodom.html>). An alignment of the "GTP-binding rhodopsin" domain (amino acids 28-212 of SEQ ID NO:2) of human 65499 with consensus amino acid sequences (SEQ ID NO:13) derived from a hidden Markov model is depicted in Figure 7. The consensus sequence for SEQ ID NO:13 is 26% identical over amino acids 28 to 212 of SEQ ID NO:2 as shown in Figure 7.

A BLAST search was performed against the HMM database resulting in the identification of regions homologous to ProDom family PD000009("Receptor coupled G-protein transmembrane glycoprotein phosphorylation lipoprotein palmitate family multigene" SEQ ID NO:15, ProDomain Release 2001.1;

5 <http://www.toulouse.inra.fr/prodom.html>). An alignment of the "Receptor coupled G-protein transmembrane glycoprotein phosphorylation lipoprotein palmitate family multigene" domain (amino acids 65-175 of SEQ ID NO:4) of human 58875 with consensus amino acid sequences (SEQ ID NO:15) derived from a hidden Markov model is depicted in Figure 11. The consensus sequence for SEQ ID NO:15 is 32% identical over amino acids
10 65 to 175 of SEQ ID NO:4 as shown in Figure 11.

A BLAST search was performed against the HMM database resulting in the identification of regions homologous to ProDom family PD061380("Receptor SLC-1 protein-coupled G-protein transmembrane GPR24" SEQ ID NO:16, ProDomain Release 2001.1; <http://www.toulouse.inra.fr/prodom.html>). An alignment of the "Receptor SLC-1
15 protein-coupled G-protein transmembrane GPR24" domain (amino acids 270-334 of SEQ ID NO:4) of human 58875 with consensus amino acid sequences (SEQ ID NO:16) derived from a hidden Markov model is depicted in Figure 12. The consensus sequence for SEQ ID NO:16 is 38% identical over amino acids 270 to 334 of SEQ ID NO:4 as shown in Figure 12.

20 A BLAST search was performed against the HMM database resulting in the identification of regions homologous to ProDom family PD310793("Receptor orphan GPR26 protein-coupled" SEQ ID NO:17, ProDomain Release 2001.1; <http://www.toulouse.inra.fr/prodom.html>). An alignment of the "Receptor orphan GPR26 protein-coupled" domain (amino acids 235-339 of SEQ ID NO:4) of human 58875 with
25 consensus amino acid sequences (SEQ ID NO:17) derived from a hidden Markov model is depicted in Figure 13. The consensus sequence for SEQ ID NO:17 is 27% identical over amino acids 235 to 339 of SEQ ID NO:4 as shown in Figure 13.

A BLAST search was performed against the HMM database resulting in the identification of regions homologous to ProDom family PD191540("Receptor H10E21.2 protein-coupled ZC84.4 G-protein glycoprotein transmembrane" SEQ ID NO:18, ProDomain Release 2001.1; <http://www.toulouse.inra.fr/prodom.html>). An alignment of the "Receptor H10E21.2 protein-coupled ZC84.4 G-protein glycoprotein transmembrane"
30 domain (amino acids 26-189 of SEQ ID NO:4) of human 58875 with consensus amino acid

sequences (SEQ ID NO:18) derived from a hidden Markov model is depicted in Figure 14. The consensus sequence for SEQ ID NO:18 is 25% identical over amino acids 26 to 189 of SEQ ID NO:4 as shown in Figure 14.

A 65499 or 58875 polypeptide can include at least one, two, three, four, five, six, preferably seven "transmembrane domains" or regions homologous with "transmembrane domains". As used herein, the term "transmembrane domain" includes an amino acid sequence of about 10 to 40 amino acid residues in length and spans the plasma membrane. Transmembrane domains are rich in hydrophobic residues, *e.g.*, at least 50%, 60%, 70%, 80%, 90%, 95% or more of the amino acids of a transmembrane domain are hydrophobic, *e.g.*, leucines, isoleucines, tyrosines, or tryptophans. Transmembrane domains typically have alpha-helical structures and are described in, for example, Zagotta, W.N. *et al.*, (1996) *Annual Rev. Neurosci.* 19:235-263, the contents of which are incorporated herein by reference.

In a preferred embodiment, a 65499 or 58875 polypeptide or protein has at least one, two, three, four, five, six, preferably seven "transmembrane domains" or regions which includes at least about 12 to 35 more preferably about 14 to 30 or 15 to 25 amino acid residues and has at least about 60%, 70% 80% 90% 95%, 99%, or 100% homology with a "transmembrane domain," *e.g.*, the transmembrane domains of human 65499 or 58875 (*e.g.*, residues 32-56, 73-97, 106-127, 149-170, 194-218, 400-424, and 434-456 of SEQ ID NO:2 or residues 36-60, 79-90, 99-115, 153-171, 199-223, 252-270, and 288-309 of SEQ ID NO:4). The transmembrane domain of human 65499 or 58875 is visualized in the hydropathy plots (Figures 2 and 9) as regions of about 15 to 25 amino acids where the hydropathy trace is mostly above the horizontal line.

To identify the presence of a "transmembrane" domain in a 65499 or 58875 protein sequence, and make the determination that a polypeptide or protein of interest has a particular profile, the amino acid sequence of the protein can be analyzed by a transmembrane prediction method that predicts the secondary structure and topology of integral membrane proteins based on the recognition of topological models (MEMSAT, Jones *et al.*, (1994) *Biochemistry* 33:3038-3049).

A 65499 or 58875 polypeptide can include at least one, two, three, four, five, six, seven, preferably eight "non-transmembrane regions." As used herein, the term "non-transmembrane region" includes an amino acid sequence not identified as a transmembrane domain. The non-transmembrane regions in 65499 or 58875 are located at about amino

acids 1-31, 57-72, 98-105, 128-148, 171-193, 219-399, 425-433, and 457-508 of SEQ ID NO:2 or about amino acids 1-35, 61-69, 91-98, 116-152, 172-198, 224-251, 271-287, and 310-340 of SEQ ID NO:4.

The non-transmembrane regions of 65499 or 58875 include at least one, two, three, preferably four cytoplasmic regions. In one embodiment, a cytoplasmic region of a 65499 or 58875 protein can include the C-terminus and can be a "C-terminal cytoplasmic domain," also referred to herein as a "C-terminal cytoplasmic tail." As used herein, a "C-terminal cytoplasmic domain" includes an amino acid sequence having a length of at least about 10, preferably about 20 to 70, more preferably about 30 to 60 amino acid residues and is located inside of a cell or within the cytoplasm of a cell. The N-terminal amino acid residue of a "C-terminal cytoplasmic domain" is adjacent to a C-terminal amino acid residue of a transmembrane domain in a 65499 or 58875 protein. For example, a C-terminal cytoplasmic domain is located at about amino acid residues 457-508 of SEQ ID NO:2 or about amino acid residues 310-340 of SEQ ID NO:4.

In a preferred embodiment, a 65499 or 58875 polypeptide or protein has a C-terminal cytoplasmic domain or a region which includes at least about 5, preferably about 20 to 70, and more preferably about 30 to 60 amino acid residues and has at least about 60%, 70% 80% 90% 95%, 99%, or 100% homology with a C-terminal cytoplasmic domain," *e.g.*, the C-terminal cytoplasmic domain of human 65499 or 58875 (*e.g.*, residues 457-508 of SEQ ID NO:2 or residues 310-340 of SEQ ID NO:4).

In another embodiment, a 65499 or 58875 protein includes at least one, two, preferably three cytoplasmic loops. As used herein, the term "loop" includes an amino acid sequence that resides outside of a phospholipid membrane, having a length of at least about 4, preferably about 5 to 200, more preferably about 6 to 180 amino acid residues, and has an amino acid sequence that connects two transmembrane domains within a protein or polypeptide. Accordingly, the N-terminal amino acid of a loop is adjacent to a C-terminal amino acid of a transmembrane domain in a 65499 or 58875 molecule, and the C-terminal amino acid of a loop is adjacent to an N-terminal amino acid of a transmembrane domain in a 65499 or 58875 molecule. As used herein, a "cytoplasmic loop" includes a loop located inside of a cell or within the cytoplasm of a cell. For example, a "cytoplasmic loop" can be found at about amino acid residues 57-72, 128-148, and 219-399 of SEQ ID NO:2 or residues 61-69, 116-152, and 224-251 of SEQ ID NO:4.

In a preferred embodiment, a 65499 or 58875 polypeptide or protein has a cytoplasmic loop or a region which includes at least about 4, preferably about 5 to 200, and more preferably about 6 to 180 amino acid residues and has at least about 60%, 70% 80% 90% 95%, 99%, or 100% homology with a cytoplasmic loop," *e.g.*, a cytoplasmic loop of
5 human 65499 or 58875 (*e.g.*, residues 57-72, 128-148, and 219-399 of SEQ ID NO:2 or residues 61-69, 116-152, and 224-251 of SEQ ID NO:4).

In another embodiment, a 65499 or 58875 protein includes at least one, two, preferably three non-cytoplasmic loops. As used herein, a "non-cytoplasmic loop" includes an amino acid sequence located outside of a cell or within an intracellular organelle. Non-
10 cytoplasmic loops include extracellular domains (*i.e.*, outside of the cell) and intracellular domains (*i.e.*, within the cell). When referring to membrane-bound proteins found in intracellular organelles (*e.g.*, mitochondria, endoplasmic reticulum, peroxisomes microsomes, vesicles, endosomes, and lysosomes), non-cytoplasmic loops include those domains of the protein that reside in the lumen of the organelle or the matrix or the
15 intermembrane space. For example, a "non-cytoplasmic loop" can be found at about amino acid residues 98-105, 171-193, and 425-433 of SEQ ID NO:2 or about amino acids 91-98, 172-198, and 271-287 of SEQ ID NO:4.

In a preferred embodiment, a 65499 or 58875 polypeptide or protein has at least one extracellular loop or a region which includes at least about 4, preferably about 5-50,
20 preferably about 6-40, and more preferably about 7-30 amino acid residues and has at least about 60%, 70% 80% 90% 95%, 99%, or 100% homology with an "extracellular loop," *e.g.*, at least one extracellular loop of human 65499 (*e.g.*, residues 98-105, 171-193 and/or 425-433 of SEQ ID NO:2) or at least one extracellular loop of human 58875 (*e.g.*, residues 91-98, 172-198 and/or 271-287 of SEQ ID NO:4).

25 In a preferred embodiment, a 65499 family member can include at least one seven transmembrane receptor family domain (PFAM Accession Number PF00001). Furthermore, a 65499 family member can include at least one, two, and preferably three N-glycosylation sites (PS00001); at least one, and preferably two cAMP- and cGMP-dependent protein kinase phosphorylation sites (PS00004); at least one, two, three, four,
30 five, six, seven, and preferably eight protein kinase C phosphorylation sites (PS00005); at least one, two, three, four, and preferably five casein kinase II phosphorylation sites (PS00006); at least one, two, three, four, five, and preferably six N-myristoylation sites

(PS00008); at least one prokaryotic membrane lipoprotein lipid attachment site (PS00013); and at least one G-protein coupled receptors signature site (PS00237).

In a preferred embodiment, a 58875 family member can include at least one seven transmembrane receptor family domain (PFAM Accession Number PF00001).

5 Furthermore, a 58875 family member can include at least one, preferably two N-glycosylation sites (PS00001); at least one, preferably two cAMP- and cGMP-dependent protein kinase phosphorylation sites (PS00004); at least one, two, three, and preferably four protein kinase C phosphorylation sites (PS00005); at least one, two, three, four, five, and preferably six casein kinase II phosphorylation sites (PS00006); at least one, two, three,
10 four, and preferably five N-myristoylation sites (PS00008); at least one microbodies C-terminal targeting signal (PS00342); and at least one G-protein coupled receptors signature site (PS00237).

As the 65499 or 58875 polypeptides of the invention may modulate 65499- or 58875-mediated activities, they may be useful as of for developing novel diagnostic and
15 therapeutic agents for 65499- or 58875-mediated or related disorders, as described below.

Based on the above-described sequence similarities, the 65499 or 58875 molecules of the present invention are predicted to have similar biological activities as seven transmembrane receptor family members. Thus, the 65499 or 58875 molecules can act as novel diagnostic targets and therapeutic agents for controlling one or more of cellular
20 proliferative and/or differentiative disorders, disorders associated with bone metabolism, hematopoietic disorders, cardiovascular disorders, including endothelial cell disorders, blood vessel disorders, brain disorders, and hormonal disorders.

Examples of cellular proliferative and/or differentiative disorders include cancer, *e.g.*, carcinoma, sarcoma, metastatic disorders or hematopoietic neoplastic disorders, *e.g.*,
25 leukemias. A metastatic tumor can arise from a multitude of primary tumor types, including but not limited to those of prostate, colon, lung, breast and liver origin.

As used herein, the terms "cancer", "hyperproliferative" and "neoplastic" refer to cells having the capacity for autonomous growth, *i.e.*, an abnormal state or condition characterized by rapidly proliferating cell growth. Hyperproliferative and neoplastic
30 disease states may be categorized as pathologic, *i.e.*, characterizing or constituting a disease state, or may be categorized as non-pathologic, *i.e.*, a deviation from normal but not associated with a disease state. The term is meant to include all types of cancerous growths or oncogenic processes, metastatic tissues or malignantly transformed cells, tissues, or

organs, irrespective of histopathologic type or stage of invasiveness. "Pathologic hyperproliferative" cells occur in disease states characterized by malignant tumor growth. Examples of non-pathologic hyperproliferative cells include proliferation of cells associated with wound repair.

5 The terms "cancer" or "neoplasms" include malignancies of the various organ systems, such as affecting lung, breast, thyroid, lymphoid, gastrointestinal, and genitourinary tract, as well as adenocarcinomas which include malignancies such as most colon cancers, renal-cell carcinoma, prostate cancer and/or testicular tumors, non-small cell carcinoma of the lung, cancer of the small intestine and cancer of the esophagus.

10 The term "carcinoma" is art recognized and refers to malignancies of epithelial or endocrine tissues including respiratory system carcinomas, gastrointestinal system carcinomas, genitourinary system carcinomas, testicular carcinomas, breast carcinomas, prostatic carcinomas, endocrine system carcinomas, and melanomas. Exemplary carcinomas include those forming from tissue of the cervix, lung, prostate, breast, head and
15 neck, colon and ovary. The term also includes carcinosarcomas, *e.g.*, which include malignant tumors composed of carcinomatous and sarcomatous tissues. An "adenocarcinoma" refers to a carcinoma derived from glandular tissue or in which the tumor cells form recognizable glandular structures.

 The term "sarcoma" is art recognized and refers to malignant tumors of
20 mesenchymal derivation.

 Aberrant expression and/or activity of 65499 or 58875 molecules may mediate disorders associated with bone metabolism. "Bone metabolism" refers to direct or indirect effects in the formation or degeneration of bone structures, *e.g.*, bone formation, bone resorption, etc., which may ultimately affect the concentrations in serum of calcium and
25 phosphate. This term also includes activities mediated by 65499 or 58875 molecules effects in bone cells, *e.g.* osteoclasts and osteoblasts, that may in turn result in bone formation and degeneration. For example, 65499 or 58875 molecules may support different activities of bone resorbing osteoclasts such as the stimulation of differentiation of monocytes and mononuclear phagocytes into osteoclasts. Accordingly, 65499 or 58875
30 molecules that modulate the production of bone cells can influence bone formation and degeneration, and thus may be used to treat bone disorders. Examples of such disorders include, but are not limited to, osteoporosis, osteodystrophy, osteomalacia, rickets, osteitis fibrosa cystica, renal osteodystrophy, osteosclerosis, anti-convulsant treatment, osteopenia,

fibrogenesis-imperfecta ossium, secondary hyperparathyroidism, hypoparathyroidism, hyperparathyroidism, cirrhosis, obstructive jaundice, drug induced metabolism, medullary carcinoma, chronic renal disease, rickets, sarcoidosis, glucocorticoid antagonism, malabsorption syndrome, steatorrhea, tropical sprue, idiopathic hypercalcemia and milk
5 fever.

Additional examples of hematopoietic disorders or diseases include, but are not limited to, autoimmune diseases (including, for example, diabetes mellitus, arthritis (including rheumatoid arthritis, juvenile rheumatoid arthritis, osteoarthritis, psoriatic arthritis), multiple sclerosis, encephalomyelitis, myasthenia gravis, systemic lupus
10 erythematosus, autoimmune thyroiditis, dermatitis (including atopic dermatitis and eczematous dermatitis), psoriasis, Sjögren's Syndrome, Crohn's disease, aphthous ulcer, iritis, conjunctivitis, keratoconjunctivitis, ulcerative colitis, asthma, allergic asthma, cutaneous lupus erythematosus, scleroderma, vaginitis, proctitis, drug eruptions, leprosy reversal reactions, erythema nodosum leprosum, autoimmune uveitis, allergic
15 encephalomyelitis, acute necrotizing hemorrhagic encephalopathy, idiopathic bilateral progressive sensorineural hearing loss, aplastic anemia, pure red cell anemia, idiopathic thrombocytopenia, polychondritis, Wegener's granulomatosis, chronic active hepatitis, Stevens-Johnson syndrome, idiopathic sprue, lichen planus, Graves' disease, sarcoidosis, primary biliary cirrhosis, uveitis posterior, and interstitial lung fibrosis), graft-versus-host
20 disease, cases of transplantation, and allergy such as, atopic allergy.

Examples of disorders involving the heart or "cardiovascular disorder" include, but are not limited to, a disease, disorder, or state involving the cardiovascular system, *e.g.*, the heart, the blood vessels, and/or the blood. A cardiovascular disorder can be caused by an imbalance in arterial pressure, a malfunction of the heart, or an occlusion of a blood vessel,
25 *e.g.*, by a thrombus. Examples of cardiovascular disorders include but are not limited to, hypertension, atherosclerosis, coronary artery spasm, coronary artery disease, arrhythmias, heart failure, including but not limited to, cardiac hypertrophy, left-sided heart failure, and right-sided heart failure; ischemic heart disease, including but not limited to angina pectoris, myocardial infarction, chronic ischemic heart disease, and sudden cardiac death;
30 hypertensive heart disease, including but not limited to, systemic (left-sided) hypertensive heart disease and pulmonary (right-sided) hypertensive heart disease; valvular heart disease, including but not limited to, valvular degeneration caused by calcification, such as calcification of a congenitally bicuspid aortic valve, and mitral annular calcification, and

myxomatous degeneration of the mitral valve (mitral valve prolapse), rheumatic fever and rheumatic heart disease, infective endocarditis, and noninfected vegetations, such as nonbacterial thrombotic endocarditis and endocarditis of systemic lupus erythematosus (Libman-Sacks disease), carcinoid heart disease, and complications of artificial valves;

5 myocardial disease, including but not limited to dilated cardiomyopathy, hypertrophic cardiomyopathy, restrictive cardiomyopathy, and myocarditis; pericardial disease, including but not limited to, pericardial effusion and hemopericardium and pericarditis, including acute pericarditis and healed pericarditis, and rheumatoid heart disease; neoplastic heart disease, including but not limited to, primary cardiac tumors, such as myxoma, lipoma,

10 papillary fibroelastoma, rhabdomyoma, and sarcoma, and cardiac effects of noncardiac neoplasms; congenital heart disease, including but not limited to, left-to-right shunts--late cyanosis, such as atrial septal defect, ventricular septal defect, patent ductus arteriosus, and atrioventricular septal defect, right-to-left shunts--early cyanosis, such as tetralogy of fallot, transposition of great arteries, truncus arteriosus, tricuspid atresia, and total anomalous

15 pulmonary venous connection, obstructive congenital anomalies, such as coarctation of aorta, pulmonary stenosis and atresia, and aortic stenosis and atresia, disorders involving cardiac transplantation, and congestive heart failure.

A cardiovascular disease or disorder also includes an endothelial cell disorder. As used herein, an "endothelial cell disorder" includes a disorder characterized by aberrant,

20 unregulated, or unwanted endothelial cell activity, *e.g.*, proliferation, migration, angiogenesis, or vascularization; or aberrant expression of cell surface adhesion molecules or genes associated with angiogenesis, *e.g.*, TIE-2, FLT and FLK. Endothelial cell disorders include tumorigenesis, tumor metastasis, psoriasis, diabetic retinopathy, endometriosis, Grave's disease, ischemic disease (*e.g.*, atherosclerosis), and chronic

25 inflammatory diseases (*e.g.*, rheumatoid arthritis).

Disorders involving the brain include, but are not limited to, disorders involving neurons, and disorders involving glia, such as astrocytes, oligodendrocytes, ependymal cells, and microglia; cerebral edema, raised intracranial pressure and herniation, and hydrocephalus; malformations and developmental diseases, such as neural tube defects,

30 forebrain anomalies, posterior fossa anomalies, and syringomyelia and hydromyelia; perinatal brain injury; cerebrovascular diseases, such as those related to hypoxia, ischemia, and infarction, including hypotension, hypoperfusion, and low-flow states--global cerebral ischemia and focal cerebral ischemia--infarction from obstruction of local blood supply,

intracranial hemorrhage, including intracerebral (intraparenchymal) hemorrhage, subarachnoid hemorrhage and ruptured berry aneurysms, and vascular malformations, hypertensive cerebrovascular disease, including lacunar infarcts, slit hemorrhages, and hypertensive encephalopathy; infections, such as acute meningitis, including acute

5 pyogenic (bacterial) meningitis and acute aseptic (viral) meningitis, acute focal suppurative infections, including brain abscess, subdural empyema, and extradural abscess, chronic bacterial meningoencephalitis, including tuberculosis and mycobacterioses, neurosyphilis, and neuroborreliosis (Lyme disease), viral meningoencephalitis, including arthropod-borne (Arbo) viral encephalitis, *Herpes simplex* virus Type 1, *Herpes simplex* virus Type 2,

10 *Varicella-zoster* virus (*Herpes zoster*), cytomegalovirus, poliomyelitis, rabies, and human immunodeficiency virus 1, including HIV-1 meningoencephalitis (subacute encephalitis), vacuolar myelopathy, AIDS-associated myopathy, peripheral neuropathy, and AIDS in children, progressive multifocal leukoencephalopathy, subacute sclerosing panencephalitis, fungal meningoencephalitis, other infectious diseases of the nervous system; transmissible

15 spongiform encephalopathies (prion diseases); demyelinating diseases, including multiple sclerosis, multiple sclerosis variants, acute disseminated encephalomyelitis and acute necrotizing hemorrhagic encephalomyelitis, and other diseases with demyelination; degenerative diseases, such as degenerative diseases affecting the cerebral cortex, including Alzheimer disease and Pick disease, degenerative diseases of basal ganglia and brain stem,

20 including Parkinsonism, idiopathic Parkinson disease (paralysis agitans), progressive supranuclear palsy, corticobasal degeneration, multiple system atrophy, including striatonigral degeneration, Shy-Drager syndrome, and olivopontocerebellar atrophy, and Huntington disease; spinocerebellar degenerations, including spinocerebellar ataxias, including Friedreich ataxia, and ataxia-telangiectasia, degenerative diseases affecting motor

25 neurons, including amyotrophic lateral sclerosis (motor neuron disease), bulbospinal atrophy (Kennedy syndrome), and spinal muscular atrophy; inborn errors of metabolism, such as leukodystrophies, including Krabbe disease, metachromatic leukodystrophy, adrenoleukodystrophy, Pelizaeus-Merzbacher disease, and Canavan disease, mitochondrial encephalomyopathies, including Leigh disease and other mitochondrial

30 encephalomyopathies; toxic and acquired metabolic diseases, including vitamin deficiencies such as thiamine (vitamin B₁) deficiency and vitamin B₁₂ deficiency, neurologic sequelae of metabolic disturbances, including hypoglycemia, hyperglycemia, and hepatic encephatopathy, toxic disorders, including carbon monoxide, methanol,

ethanol, and radiation, including combined methotrexate and radiation-induced injury; tumors, such as gliomas, including astrocytoma, including fibrillary (diffuse) astrocytoma and glioblastoma multiforme, pilocytic astrocytoma, pleomorphic xanthoastrocytoma, and brain stem glioma, oligodendroglioma, and ependymoma and related paraventricular mass lesions, neuronal tumors, poorly differentiated neoplasms, including medulloblastoma, other parenchymal tumors, including primary brain lymphoma, germ cell tumors, and pineal parenchymal tumors, meningiomas, metastatic tumors, paraneoplastic syndromes, peripheral nerve sheath tumors, including schwannoma, neurofibroma, and malignant peripheral nerve sheath tumor (malignant schwannoma), and neurocutaneous syndromes (phakomatoses), including neurofibromatosis, including Type 1 neurofibromatosis (NF1) and TYPE 2 neurofibromatosis (NF2), tuberous sclerosis, and Von Hippel-Lindau disease.

Hormonal disorders include such conditions or diseases in which the production and/or regulation of hormones in an organism is aberrant. Examples of such disorders and diseases include type I and type II diabetes mellitus, pituitary disorders (*e.g.*, growth disorders), thyroid disorders (*e.g.*, hypothyroidism or hyperthyroidism), and reproductive or fertility disorders (*e.g.*, disorders which affect the organs of the reproductive system, *e.g.*, the prostate gland, the uterus, or the vagina; disorders which involve an imbalance in the levels of a reproductive hormone in a subject; disorders affecting the ability of a subject to reproduce; and disorders affecting secondary sex characteristic development, *e.g.*, adrenal hyperplasia).

The 65499 or 58875 nucleic acid and protein of the invention can be used to treat and/or diagnose a variety of immune disorders. Exemplary immune disorders include hematopoietic neoplastic disorders. As used herein, the term "hematopoietic neoplastic disorders" includes diseases involving hyperplastic/neoplastic cells of hematopoietic origin, *e.g.*, arising from myeloid, lymphoid or erythroid lineages, or precursor cells thereof. Preferably, the diseases arise from poorly differentiated acute leukemias, *e.g.*, erythroblastic leukemia and acute megakaryoblastic leukemia. Additional exemplary myeloid disorders include, but are not limited to, acute promyeloid leukemia (APML), acute myelogenous leukemia (AML) and chronic myelogenous leukemia (CML) (reviewed in Vaickus, L. (1991) *Crit Rev. in Oncol./Hematol.* 11:267-97); lymphoid malignancies include, but are not limited to acute lymphoblastic leukemia (ALL) which includes B-lineage ALL and T-lineage ALL, chronic lymphocytic leukemia (CLL), prolymphocytic leukemia (PLL), hairy cell leukemia (HLL) and Waldenstrom's macroglobulinemia (WM). Additional forms of

malignant lymphomas include, but are not limited to non-Hodgkin lymphoma and variants thereof, peripheral T cell lymphomas, adult T cell leukemia/lymphoma (ATL), cutaneous T-cell lymphoma (CTCL), large granular lymphocytic leukemia (LGF), Hodgkin's disease and Reed-Sternberg disease.

5' Disorders which may be treated or diagnosed by methods described herein include, but are not limited to, disorders associated with an accumulation in the liver of fibrous tissue, such as that resulting from an imbalance between production and degradation of the extracellular matrix accompanied by the collapse and condensation of preexisting fibers. The methods described herein can be used to diagnose or treat hepatocellular necrosis or
10 injury induced by a wide variety of agents including processes which disturb homeostasis, such as an inflammatory process, tissue damage resulting from toxic injury or altered hepatic blood flow, and infections (e.g., bacterial, viral and parasitic). For example, the methods can be used for the early detection of hepatic injury, such as portal hypertension or hepatic fibrosis. In addition, the methods can be employed to detect liver fibrosis attributed
15 to inborn errors of metabolism, for example, fibrosis resulting from a storage disorder such as Gaucher's disease (lipid abnormalities) or a glycogen storage disease, A1-antitrypsin deficiency; a disorder mediating the accumulation (e.g., storage) of an exogenous substance, for example, hemochromatosis (iron-overload syndrome) and copper storage diseases (Wilson's disease), disorders resulting in the accumulation of a toxic metabolite
20 (e.g., tyrosinemia, fructosemia and galactosemia) and paroxysmal disorders (e.g., Zellweger syndrome). Additionally, the methods described herein may be useful for the early detection and treatment of liver injury associated with the administration of various chemicals or drugs, such as for example, methotrexate, isoniazid, oxyphenisatin, methyl dopa, chlorpromazine, tolbutamide or alcohol, or which represents a hepatic
25 manifestation of a vascular disorder such as obstruction of either the intrahepatic or extrahepatic bile flow or an alteration in hepatic circulation resulting, for example, from chronic heart failure, veno-occlusive disease, portal vein thrombosis or Budd-Chiari syndrome.

Additionally, 65499 or 58875 molecules may play an important role in the etiology
30 of certain viral diseases, including but not limited to Hepatitis B, Hepatitis C and Herpes Simplex Virus (HSV). Modulators of 65499 or 58875 activity could be used to control viral diseases. The modulators can be used in the treatment and/or diagnosis of viral infected tissue or virus-associated tissue fibrosis, especially liver and liver fibrosis. Also,

65499 or 58875 modulators can be used in the treatment and/or diagnosis of virus-associated carcinoma, especially hepatocellular cancer.

Additionally, 65499 or 58875 may play an important role in the regulation of metabolism or, in a preferred embodiment, pain disorders. Diseases of metabolic imbalance include, but are not limited to, obesity, anorexia nervosa, cachexia, lipid disorders diabetes. Examples of pain disorders include, but are not limited to, pain response elicited during various forms of tissue injury, *e.g.*, inflammation, infection, and ischemia, usually referred to as hyperalgesia (described in, for example, Fields, H. L. (1987) *Pain*, New York:McGraw-Hill); pain associated with musculoskeletal disorders, *e.g.*, joint pain; tooth pain; headaches; pain associated with surgery; pain related to irritable bowel syndrome; or chest pain.

The 65499 or 58875 protein, fragments thereof, and derivatives and other variants of the sequences in SEQ ID NO:2 and SEQ ID NO:4, thereof are collectively referred to as "polypeptides or proteins of the invention" or "65499 or 58875 polypeptides or proteins". Nucleic acid molecules encoding such polypeptides or proteins are collectively referred to as "nucleic acids of the invention" or "65499 or 58875 nucleic acids." 65499 or 58875 molecules refer to 65499 or 58875 nucleic acids, polypeptides, and antibodies.

As used herein, the term "nucleic acid molecule" includes DNA molecules (*e.g.*, a cDNA or genomic DNA) and RNA molecules (*e.g.*, an mRNA) and analogs of the DNA or RNA generated, *e.g.*, by the use of nucleotide analogs. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

The term "isolated or purified nucleic acid molecule" includes nucleic acid molecules which are separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. For example, with regards to genomic DNA, the term "isolated" includes nucleic acid molecules which are separated from the chromosome with which the genomic DNA is naturally associated. Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (*i.e.*, sequences located at the 5' and/or 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated nucleic acid molecule can contain less than about 5 kb, 4kb, 3kb, 2kb, 1 kb, 0.5 kb or 0.1 kb of 5' and/or 3' nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or

culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized.

As used herein, the term "hybridizes under stringent conditions" describes conditions for hybridization and washing. Stringent conditions are known to those skilled in the art and can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. Aqueous and nonaqueous methods are described in that reference and either can be used. A preferred, example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 50°C. Another example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 55°C. A further example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 60°C. Preferably, stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 65°C. Particularly preferred stringency conditions (and the conditions that should be used if the practitioner is uncertain about what conditions should be applied) are 0.5M Sodium Phosphate, 7% SDS at 65°C, followed by one or more washes at 0.2X SSC, 1% SDS at 65°C. Preferably, an isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequence of SEQ ID NO:1, 3, 5 and/or 6 corresponds to a naturally-occurring nucleic acid molecule.

As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (e.g., encodes a natural protein).

As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules which include an open reading frame encoding a 65499 or 58875 protein, preferably a mammalian 65499 or 58875 protein, and can further include non-coding regulatory sequences, and introns.

An "isolated" or "purified" polypeptide or protein is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. In one embodiment, the language "substantially free" means

preparation of 65499 or 58875 protein having less than about 30%, 20%, 10% and more preferably 5% (by dry weight), of non-65499 or non-58875 protein (also referred to herein as a "contaminating protein"), or of chemical precursors or non-65499 or non-58875 chemicals. When the 65499 or 58875 protein or biologically active portion thereof is
5 recombinantly produced, it is also preferably substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the protein preparation. The invention includes isolated or purified preparations of at least 0.01, 0.1, 1.0, and 10 milligrams in dry weight.

10 A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of 65499 or 58875 (*e.g.*, the sequence of SEQ ID NO:1, 3, 5, or 6, or the nucleotide sequence of the DNA insert of one of the plasmids deposited with ATCC as Accession Numbers _____) without abolishing or more preferably, without substantially altering a biological activity, whereas an "essential" amino acid residue results in such a
15 change. For example, amino acid residues that are conserved among the polypeptides of the present invention, *e.g.*, those present in the seven transmembrane receptor domains, are predicted to be particularly unamenable to alteration.

A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid
20 residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side
25 chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in a 65499 or 58875 protein is preferably replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of a 65499 or 58875 coding sequence, such as by saturation
30 mutagenesis, and the resultant mutants can be screened for 65499 or 58875 biological activity to identify mutants that retain activity. Following mutagenesis of SEQ ID NO:1, 3, 5 or 6, or the nucleotide sequence of the DNA insert of one of the plasmids deposited with

ATCC as Accession Numbers _____), the encoded protein can be expressed recombinantly and the activity of the protein can be determined.

As used herein, a "biologically active portion" of a 65499 or 58875 protein includes a fragment of a 65499 or 58875 protein which participates in an interaction
5 between a 65499 or 58875 molecule and a non-65499 or 58875 molecule. Biologically active portions of a 65499 or 58875 protein include peptides comprising amino acid sequences sufficiently homologous to or derived from the amino acid sequence of the 65499 or 58875 protein, *e.g.*, the amino acid sequence shown in SEQ ID NO:2 or SEQ ID NO:4, which include less amino acids than the full length 65499 or 58875 proteins, and
10 exhibit at least one activity of a 65499 or 58875 protein. Typically, biologically active portions comprise a domain or motif with at least one activity of the 65499 or 58875 protein, *e.g.*, a domain or motif capable of regulating, sensing and/or transmitting an extracellular signal into a cell; a domain or motif capable of interacting with (*e.g.*, binding to) an extracellular signal or a cell surface receptor; a domain or motif capable of
15 mobilizing an intracellular molecule that participates in a signal transduction pathway (*e.g.*, adenylate cyclase or phosphatidylinositol 4,5-bisphosphate (PIP₂), inositol 1,4,5-triphosphate (IP₃)); a domain or motif capable of regulating polarization of the plasma membrane; a domain or motif capable of controlling production or secretion of molecules; a domain or motif capable of altering the structure of a cellular component; a domain or motif
20 capable of modulating cell proliferation, *e.g.*, synthesis of DNA; and/or a domain or motif capable of modulating cell migration, cell differentiation; and/or cell survival.

A biologically active portion of a 65499 or 58875 protein can be a polypeptide which is, for example, 10, 25, 50, 100, 200 or more amino acids in length. Biologically active portions of a 65499 or 58875 protein can be used as targets for developing agents
25 which modulate a 65499 or 58875 mediated activity, *e.g.*, a biological activity described herein.

Calculations of homology or sequence identity between sequences (the terms are used interchangeably herein) are performed as follows.

To determine the percent identity of two amino acid sequences, or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can
30 be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison

purposes). In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, 90%, 100% of the length of the reference sequence (*e.g.*, when aligning a second sequence to the 5 65499 amino acid sequence of SEQ ID NO:2, having 508 amino acid residues, at least 152, preferably at least 203, more preferably at least 254, even more preferably at least 305, and even more preferably at least 357, 406, 457, or 508 amino acid residues are aligned; or when aligning a second sequence to the 58875 amino acid sequence of SEQ ID NO:4, having 340 amino acid residues, at least 102, preferably at least 136, more preferably at 10 least 170, even more preferably at least 204, and even more preferably at least 238, 272, 306, or 340 amino acid residues are aligned). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that 15 position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

20 The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch (*J. Mol. Biol.* (48):444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at 25 <http://www.gcg.com>), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available at <http://www.gcg.com>), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, 30 or 80 and a length weight of 1, 2, 3, 4, 5, or 6. A particularly preferred set of parameters (and the one that should be used if the practitioner is uncertain about what parameters should be applied to determine if a molecule is within the invention) is using a Blossum 62

scoring matrix with a gap open penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5.

The percent identity between two amino acid or nucleotide sequences can be determined using the algorithm of E. Meyers and W. Miller (CABIOS, 4:11-17 (1989)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

The nucleic acid and protein sequences described herein can be used as a "query sequence" to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, *et al.* (1990) *J. Mol. Biol.* 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, word length = 12 to obtain nucleotide sequences homologous to 65499 or 58875 nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, word length = 3 to obtain amino acid sequences homologous to 65499 or 58875 protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, (1997) *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used. See <http://www.ncbi.nlm.nih.gov>.

Particular 65499 or 58875 polypeptides of the present invention have an amino acid sequence substantially identical to the amino acid sequence of SEQ ID NO:2 or 4. In the context of an amino acid sequence, the term "substantially identical" is used herein to refer to a first amino acid that contains a sufficient or minimum number of amino acid residues that are i) identical to, or ii) conservative substitutions of aligned amino acid residues in a second amino acid sequence such that the first and second amino acid sequences can have a common structural domain and/or common functional activity. For example, amino acid sequences that contain a common structural domain having at least about 60%, or 65% identity, likely 75% identity, more likely 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO:2 or 4 are termed substantially identical.

In the context of nucleotide sequence, the term "substantially identical" is used herein to refer to a first nucleic acid sequence that contains a sufficient or minimum number of nucleotides that are identical to aligned nucleotides in a second nucleic acid sequence such that the first and second nucleotide sequences encode a polypeptide having common

functional activity, or encode a common structural polypeptide domain or a common functional polypeptide activity. For example, nucleotide sequences having at least about 60%, or 65% identity, likely 75% identity, more likely 85%, 90%. 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO:1 or 3 are termed substantially
5 identical.

“Misexpression or aberrant expression”, as used herein, refers to a non-wild type pattern of gene expression, at the RNA or protein level. It includes: expression at non-wild type levels, *i.e.*, over or under expression; a pattern of expression that differs from wild type in terms of the time or stage at which the gene is expressed, *e.g.*, increased or
10 decreased expression (as compared with wild type) at a predetermined developmental period or stage; a pattern of expression that differs from wild type in terms of decreased expression (as compared with wild type) in a predetermined cell type or tissue type; a pattern of expression that differs from wild type in terms of the splicing size, amino acid sequence, post-translational modification, or biological activity of the expressed
15 polypeptide; a pattern of expression that differs from wild type in terms of the effect of an environmental stimulus or extracellular stimulus on expression of the gene, *e.g.*, a pattern of increased or decreased expression (as compared with wild type) in the presence of an increase or decrease in the strength of the stimulus.

“Subject”, as used herein, can refer to a mammal, *e.g.*, a human, or to an
20 experimental or animal or disease model. The subject can also be a non-human animal, *e.g.*, a horse, cow, goat, or other domestic animal.

A “purified preparation of cells”, as used herein, refers to, in the case of plant or animal cells, an *in vitro* preparation of cells and not an entire intact plant or animal. In the case of cultured cells or microbial cells, it consists of a preparation of at least 10% and
25 more preferably 50% of the subject cells.

Various aspects of the invention are described in further detail below.

Isolated Nucleic Acid Molecules

In one aspect, the invention provides, an isolated or purified, nucleic acid molecule
30 that encodes a 65499 or 58875 polypeptide described herein, *e.g.*, a full length 65499 or 58875 protein or a fragment thereof, *e.g.*, a biologically active portion of 65499 or 58875 protein. Also included is a nucleic acid fragment suitable for use as a hybridization probe, which can be used, *e.g.*, to identify nucleic acid molecule encoding a polypeptide of the

invention, 65499 or 58875 mRNA, and fragments suitable for use as primers, *e.g.*, PCR primers for the amplification or mutation of nucleic acid molecules.

In one embodiment, an isolated nucleic acid molecule of the invention includes the nucleotide sequence shown in SEQ ID NO:1, 3, 5 or 6, or the nucleotide sequence of the DNA insert of any of the plasmids deposited with ATCC as Accession Numbers _____, or a portion of any of these nucleotide sequences. In one embodiment, the nucleic acid molecule includes sequences encoding the human 65499 or 58875 protein (*i.e.*, "the coding region" of about 1527 nucleotides, of SEQ ID NO:1 or "the coding region" of about 1023 nucleotides of SEQ ID NO: 3, respectively, including termination codon), as well as 5' untranslated sequences (shown in SEQ ID NO:1 and 3), and/or the 3' untranslated (shown in SEQ ID NO:1 and 3). Alternatively, the nucleic acid molecule can include only the coding region shown in SEQ ID NO:5 and 6, and, *e.g.*, no flanking sequences which normally accompany the subject sequence. In another embodiment, the nucleic acid molecule encodes a sequence corresponding to the human 65499 or 58875 protein shown in SEQ ID NO:2 and 4, respectively.

In another embodiment, an isolated nucleic acid molecule of the invention includes a nucleic acid molecule which is a complement of the nucleotide sequence shown in SEQ ID NO:1, 3, 5, 6 or the nucleotide sequence of the DNA insert of any of the plasmids deposited with ATCC as Accession Numbers _____, or a portion of any of these nucleotide sequences. In other embodiments, the nucleic acid molecule of the invention is sufficiently complementary to the nucleotide sequence shown in SEQ ID NO:1, 3, 5, or 6, or the nucleotide sequence of the DNA insert of any of the plasmids deposited with ATCC as Accession Numbers _____, thereby forming a stable duplex.

In one embodiment, an isolated nucleic acid molecule of the present invention includes a nucleotide sequence which is at least about 60%, 65%, 70%, 75%, 78%, 79%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more homologous to the nucleotide sequence shown in SEQ ID NO:1, 3, 5, 6, or the nucleotide sequence of the DNA insert of any of the plasmids deposited with ATCC as Accession Numbers _____. In the case of an isolated nucleic acid molecule which is longer than or equivalent in length to the reference sequence, *e.g.*, SEQ ID NO:1, 3, 5 or 6, the comparison is made with the full length of the reference sequence. Where the isolated nucleic acid molecule is shorter than the reference sequence, *e.g.*, shorter than SEQ ID NO:1, 3, 5 or 6, the comparison is

made to a segment of the reference sequence of the same length (excluding any loop required by the homology calculation).

65499 or 58875 Nucleic Acid Fragments

5 A nucleic acid molecule of the invention can include only a portion of the nucleic acid sequence of SEQ ID NO:1, 3, 5, 6, or the nucleotide sequence of the DNA insert of any of the plasmids deposited with ATCC as Accession Numbers _____. For example, such a nucleic acid molecule can include a fragment which can be used as a probe or primer or a fragment encoding a portion of a 65499 or 58875 protein, *e.g.*, an immunogenic
10 or biologically active portion of a 65499 or 58875 protein. A fragment can comprise nucleotides which encode the N- and the C-termini, respectively, of human 65499 or 58875. Alternatively, the fragment can include nucleotides which encode a seven transmembrane receptor domain of human 65499 or 58875. The nucleotide sequence determined from the cloning of the 65499 or 58875 gene allows for the generation of
15 probes and primers designed for use in identifying and/or cloning other 65499 or 58875 family members, or fragments thereof, as well as 65499 or 58875 homologues, or fragments thereof, from other species.

 In another embodiment, a nucleic acid includes a nucleotide sequence that includes part, or all, of the coding region and extends into either (or both) the 5' or 3' noncoding region.
20 Other embodiments include a fragment which includes a nucleotide sequence encoding an amino acid fragment described herein. Nucleic acid fragments can encode a specific domain or site described herein or fragments thereof, particularly fragments thereof which are at least 15-25 amino acids in length. Fragments also include nucleic acid sequences corresponding to specific amino acid sequences described above or fragments thereof. Nucleic acid fragments
25 should not be construed as encompassing those fragments that may have been disclosed prior to the invention.

 A nucleic acid fragment can include a sequence corresponding to a domain, region, or functional site described herein. A nucleic acid fragment can also include one or more domain, region, or functional site described herein. Thus, for example, a 65499 or 58875
30 nucleic acid fragment can include a sequence corresponding to a seven transmembrane receptor domain, as described herein.

 65499 or 58875 probes and primers are provided. Typically a probe/primer is an isolated or purified oligonucleotide. The oligonucleotide typically includes a region of

nucleotide sequence that hybridizes under stringent conditions to at least about 7, 12 or 15, preferably about 20 or 25, more preferably about 30, 35, 40, 45, 50, 55, 60, 65, 75, 100, 150 or 200 consecutive nucleotides of a sense or antisense sequence of SEQ ID NO:1, 3, 5, 6 or the nucleotide sequence of the DNA insert of any of the plasmids deposited with ATCC as Accession Numbers _____, or of a naturally occurring allelic variant or mutant of SEQ ID NO:1, 3, 5, 6 or the nucleotide sequence of the DNA insert of any of the plasmids deposited with ATCC as Accession Numbers _____.

In a preferred embodiment the nucleic acid is a probe which is at least 5 or 10, and less than 200, more preferably less than 100, or less than 50, base pairs in length. It should be identical, or differ by 1, or less than in 5 or 10 bases, from a sequence disclosed herein. If alignment is needed for this comparison the sequences should be aligned for maximum homology. "Looped" out sequences from deletions or insertions, or mismatches, are considered differences.

A probe or primer can be derived from the sense or anti-sense strand of a nucleic acid which encodes a seven transmembrane domain of SEQ ID NO:2 or 4.

In another embodiment a set of primers is provided, *e.g.*, primers suitable for use in a PCR, which can be used to amplify a selected region of a 65499 or 58875 sequence. The primers should be at least 5, 10, or 50 base pairs in length and less than 100, or less than 200, base pairs in length. The primers should be identical, or differs by one base from a sequence disclosed herein or from a naturally occurring variant. *E.g.*, primers suitable for amplifying all or a portion of a seven transmembrane domain of SEQ ID NO:2 or 4.

A nucleic acid fragment can encode an epitope bearing region of a polypeptide described herein.

A nucleic acid fragment encoding a "biologically active portion of a 65499 or 58875 polypeptide" can be prepared by isolating a portion of the nucleotide sequence of SEQ ID NO:1, 3, 5, 6 or the nucleotide sequence of the DNA insert of any of the plasmids deposited with ATCC as Accession Numbers _____, which encodes a polypeptide having a 65499 or 58875 biological activity (*e.g.*, the biological activities of the 65499 or 58875 proteins are described herein), expressing the encoded portion of the 65499 or 58875 protein (*e.g.*, by recombinant expression *in vitro*) and assessing the activity of the encoded portion of the 65499 or 58875 protein. For example, a nucleic acid fragment encoding a biologically active portion of 65499 or 58875 includes a seven transmembrane receptor domain. A nucleic acid fragment encoding a biologically active portion of a 65499 or

58875 polypeptide, may comprise a nucleotide sequence which is greater than 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or more nucleotides in length.

In one embodiment, a nucleic acid includes a nucleotide sequence which is greater than 100, 150, 200, 250, 300, 300-350, 350-400, 400-450, 450-500, 500-550, 550-600, 600-650, 650-700, 700-750, 750-800, 800-850, 850-900, 900-950, 950-1000, 1000-1050, 1050-1100, 1100-1150, 1150-1200, 1200-1250, 1250-1300, 1300-1350, 1350-1400, 1400-1450, 1450-1500, 1500-1550, 1550-1600, 1600-1650, 1650-1700, 1700-1750 or more nucleotides in length and hybridizes under stringent hybridization conditions to a nucleic acid molecule of SEQ ID NO:1, 3, 5, 6, or the nucleotide sequence of the DNA insert of any of the plasmids deposited with ATCC as Accession Numbers ____.

65499 or 58875 Nucleic Acid Variants

The invention further encompasses nucleic acid molecules that differ from the nucleotide sequence shown in SEQ ID NO:1, 3, 5, 6 or the nucleotide sequence of the DNA insert of any of the plasmids deposited with ATCC as Accession Numbers _____. Such differences can be due to degeneracy of the genetic code (and result in a nucleic acid which encodes the same 65499 or 58875 proteins as those encoded by the nucleotide sequence disclosed herein. In another embodiment, an isolated nucleic acid molecule of the invention has a nucleotide sequence encoding a protein having an amino acid sequence which differs, by at least 1, but less than 5, 10, 20, 50, or 100 amino acid residues that shown in SEQ ID NO:2 or 4. If alignment is needed for this comparison the sequences should be aligned for maximum homology. "Looped" out sequences from deletions or insertions, or mismatches, are considered differences.

Nucleic acids of the inventor can be chosen for having codons, which are preferred, or non preferred, for a particular expression system. E.g., the nucleic acid can be one in which at least one codon, and preferably at least 10%, or 20% of the codons has been altered such that the sequence is optimized for expression in *E. coli*, yeast, human, insect, or CHO cells.

Nucleic acid variants can be naturally occurring, such as allelic variants (same locus), homologs (different locus), and orthologs (different organism) or can be non naturally occurring. Non-naturally occurring variants can be made by mutagenesis techniques, including those applied to polynucleotides, cells, or organisms. The variants can contain nucleotide substitutions, deletions, inversions and insertions. Variation can occur in either or

both the coding and non-coding regions. The variations can produce both conservative and non-conservative amino acid substitutions (as compared in the encoded product).

In a preferred embodiment, the nucleic acid differs from that of SEQ ID NO:1, 3, 5, 6 or the nucleotide sequence of the DNA insert of any of the plasmids deposited with ATCC as Accession Numbers _____, *e.g.*, as follows: by at least one but less than 10, 20, 30, or 40 nucleotides; at least one but less than 1%, 5%, 10% or 20% of the nucleotides in the subject nucleic acid. If necessary for this analysis the sequences should be aligned for maximum homology. "Looped" out sequences from deletions or insertions, or mismatches, are considered differences.

Orthologs, homologs, and allelic variants can be identified using methods known in the art. These variants comprise a nucleotide sequence encoding a polypeptide that is 50%, at least about 55%, typically at least about 70-75%, more typically at least about 80-85%, and most typically at least about 90-95% or more identical to the polypeptide sequence shown in SEQ ID NO:2 or 4 or a fragment of these sequences. Such nucleic acid molecules can readily be identified as being able to hybridize under stringent conditions, to the nucleotide sequence shown in SEQ ID NO:1, 3, 5 or 6, or a fragment of these sequences. Nucleic acid molecules corresponding to orthologs, homologs, and allelic variants of the 65499 or 58875 cDNAs of the invention can further be isolated by mapping to the same chromosome or locus as the 65499 or 58875 gene. Preferred variants include those that are correlated with any of the 65499 or 58875 biological activities described herein, *e.g.*, regulating, sensing and/or transmitting an extracellular signal into a cell; interacting with (*e.g.*, binding to) an extracellular signal or a cell surface receptor; mobilizing an intracellular molecule that participates in a signal transduction pathway; regulating polarization of the plasma membrane; controlling production or secretion of molecules; altering the structure of a cellular component; modulating cell proliferation, *e.g.*, synthesis of DNA; and modulating cell migration, cell differentiation; and cell survival.

Allelic variants of 65499 or 58875, *e.g.*, human 65499 or 58875, include both functional and non-functional proteins. Functional allelic variants are naturally occurring amino acid sequence variants of the 65499 or 58875 protein within a population that maintain the ability to mediate any of the 65499 or 58875 biological activities described herein, *e.g.*, regulating, sensing and/or transmitting an extracellular signal into a cell; interacting with (*e.g.*, binding to) an extracellular signal or a cell surface receptor; mobilizing an intracellular molecule that participates in a signal transduction pathway;

regulating polarization of the plasma membrane; controlling production or secretion of molecules; altering the structure of a cellular component; modulating cell proliferation, *e.g.*, synthesis of DNA; and modulating cell migration, cell differentiation; and cell survival.

Functional allelic variants will typically contain only conservative substitution of one or more amino acids of SEQ ID NO:2 or 4, or substitution, deletion or insertion of non-critical residues in non-critical regions of the protein. Non-functional allelic variants are naturally-occurring amino acid sequence variants of the 65499 or 58875, *e.g.*, human 65499 or 58875, protein within a population that do not have the ability to mediate any of the 65499 or 58875 biological activities described herein. Non-functional allelic variants will typically contain a non-conservative substitution, a deletion, or insertion, or premature truncation of the amino acid sequence of SEQ ID NO:2 or 4, or a substitution, insertion, or deletion in critical residues or critical regions of the protein.

Moreover, nucleic acid molecules encoding other 65499 or 58875 family members and, thus, which have a nucleotide sequence which differs from the 65499 or 58875 sequences of SEQ ID NO:1, 3, 5, 6 or the nucleotide sequence of the DNA insert of any of the plasmids deposited with ATCC as Accession Numbers _____ are intended to be within the scope of the invention.

Antisense Nucleic Acid Molecules, Ribozymes and Modified 65499 or 58875

Nucleic Acid Molecules

In another aspect, the invention features, an isolated nucleic acid molecule which is antisense to 65499 or 58875. An "antisense" nucleic acid can include a nucleotide sequence which is complementary to a "sense" nucleic acid encoding a protein, *e.g.*, complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. The antisense nucleic acid can be complementary to an entire 65499 or 58875 coding strand, or to only a portion thereof (*e.g.*, the coding region of human 65499 or 58875 corresponding to SEQ ID NO:5 and 6, respectively). In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding 65499 or 58875 (*e.g.*, the 5' and 3' untranslated regions).

An antisense nucleic acid can be designed such that it is complementary to the entire coding region of 65499 or 58875 mRNA, but more preferably is an oligonucleotide which is antisense to only a portion of the coding or noncoding region of 65499 or 58875

mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of 65499 or 58875 mRNA, *e.g.*, between the -10 and +10 regions of the target gene nucleotide sequence of interest. An antisense oligonucleotide can be, for example, about 7, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, or more nucleotides in length.

An antisense nucleic acid of the invention can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (*e.g.*, an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, *e.g.*, phosphorothioate derivatives and acridine substituted nucleotides can be used. The antisense nucleic acid also can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (*i.e.*, RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

The antisense nucleic acid molecules of the invention are typically administered to a subject (*e.g.*, by direct injection at a tissue site), or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a 65499 or 58875 protein to thereby inhibit expression of the protein, *e.g.*, by inhibiting transcription and/or translation. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, *e.g.*, by linking the antisense nucleic acid molecules to peptides or antibodies which bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of the antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

In yet another embodiment, the antisense nucleic acid molecule of the invention is an α -anomeric nucleic acid molecule. An α -anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β -units, the strands run parallel to each other (Gaultier *et al.* (1987) *Nucleic Acids. Res.* 15:6625-

6641). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (Inoue *et al.* (1987) *Nucleic Acids Res.* 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue *et al.* (1987) *FEBS Lett.* 215:327-330).

In still another embodiment, an antisense nucleic acid of the invention is a
5 ribozyme. A ribozyme having specificity for a 65499- or 58875-encoding nucleic acid can include one or more sequences complementary to the nucleotide sequence of a 65499 or 58875 cDNA disclosed herein (*i.e.*, SEQ ID NO:1, 3, 5 or 6), and a sequence having known catalytic sequence responsible for mRNA cleavage (see U.S. Pat. No. 5,093,246 or Haselhoff and Gerlach (1988) *Nature* 334:585-591). For example, a derivative of a
10 *Tetrahymena* L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a 65499- or 58875-encoding mRNA. See, *e.g.*, Cech *et al.* U.S. Patent No. 4,987,071; and Cech *et al.* U.S. Patent No. 5,116,742. Alternatively, 65499 or 58875 mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See,
15 *e.g.*, Bartel, D. and Szostak, J.W. (1993) *Science* 261:1411-1418.

65499 or 58875 gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the 65499 or 58875 (*e.g.*, the 65499 or 58875 promoter and/or enhancers) to form triple helical structures that prevent transcription of the 65499 or 58875 gene in target cells. See generally, Helene, C. (1991) *Anticancer Drug*
20 *Des.* 6(6):569-84; Helene, C. *et al.* (1992) *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher, L. J. (1992) *Bioassays* 14(12):807-15. The potential sequences that can be targeted for triple helix formation can be increased by creating a so called "switchback" nucleic acid molecule. Switchback molecules are synthesized in an alternating 5'-3', 3'-5' manner, such that they base pair with first one strand of a duplex and then the other, eliminating the
25 necessity for a sizeable stretch of either purines or pyrimidines to be present on one strand of a duplex.

The invention also provides detectably labeled oligonucleotide primer and probe molecules. Typically, such labels are chemiluminescent, fluorescent, radioactive, or colorimetric.

30 A 65499 or 58875 nucleic acid molecule can be modified at the base moiety, sugar moiety or phosphate backbone to improve, *e.g.*, the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acid molecules can be modified to generate peptide nucleic acids (see Hyrup B. *et al.* (1996)

Bioorganic & Medicinal Chemistry 4 (1): 5-23). As used herein, the terms "peptide nucleic acid" or "PNA" refers to a nucleic acid mimic, *e.g.*, a DNA mimic, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of a PNA can allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup B. *et al.* (1996) *supra*; Perry-O'Keefe *et al.* *Proc. Natl. Acad. Sci.* 93: 14670-675.

PNAs of 65499 or 58875 nucleic acid molecules can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, for example, inducing transcription or translation arrest or inhibiting replication. PNAs of 65499 or 58875 nucleic acid molecules can also be used in the analysis of single base pair mutations in a gene, (*e.g.*, by PNA-directed PCR clamping); as 'artificial restriction enzymes' when used in combination with other enzymes, (*e.g.*, S1 nucleases (Hyrup B. (1996) *supra*)); or as probes or primers for DNA sequencing or hybridization (Hyrup B. *et al.* (1996) *supra*; Perry-O'Keefe *supra*).

In other embodiments, the oligonucleotide may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:6553-6556; Lemaitre *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:648-652; PCT Publication No. W088/09810) or the blood-brain barrier (see, *e.g.*, PCT Publication No. W089/10134). In addition, oligonucleotides can be modified with hybridization-triggered cleavage agents (See, *e.g.*, Krol *et al.* (1988) *Bio-Techniques* 6:958-976) or intercalating agents. (See, *e.g.*, Zon (1988) *Pharm. Res.* 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, (*e.g.*, a peptide, hybridization triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent).

The invention also includes molecular beacon oligonucleotide primer and probe molecules having at least one region which is complementary to a 65499 or 58875 nucleic acid of the invention, two complementary regions one having a fluorophore and one a quencher such that the molecular beacon is useful for quantitating the presence of the 65499 or 58875 nucleic acid of the invention in a sample. Molecular beacon nucleic acids are described, for example, in Lizardi *et al.*, U.S. Patent No. 5,854,033; Nazarenko *et al.*, U.S. Patent No. 5,866,336, and Livak *et al.*, U.S. Patent 5,876,930.

Isolated 65499 or 58875 Polypeptides

In another aspect, the invention features, an isolated 65499 or 58875 protein, or fragment, *e.g.*, a biologically active portion, for use as immunogens or antigens to raise or test (or more generally to bind) anti-65499 or - 58875 antibodies. 65499 or 58875 protein can be isolated from cells or tissue sources using standard protein purification techniques. 65499 or 58875 protein or fragments thereof can be produced by recombinant DNA techniques or synthesized chemically.

Polypeptides of the invention include those which arise as a result of the existence of multiple genes, alternative transcription events, alternative RNA splicing events, and alternative translational and postranslational events. The polypeptide can be expressed in systems, *e.g.*, cultured cells, which result in substantially the same postranslational modifications present when expressed the polypeptide is expressed in a native cell, or in systems which result in the alteration or omission of postranslational modifications, *e.g.*, glycosylation or cleavage, present when expressed in a native cell.

In a preferred embodiment, a 65499 or 58875 polypeptide has one or more of the following characteristics:

- (i) it has the ability to regulate, sense and/or transmit an extracellular signal into a cell;
- (ii) it has the ability to interact with (*e.g.*, bind to) an extracellular signal or a cell surface receptor;
- (iii) it has the ability to mobilize an intracellular molecule that participates in a signal transduction pathway (*e.g.*, adenylate cyclase or phosphatidylinositol 4,5-bisphosphate (PIP₂), inositol 1,4,5-triphosphate (IP₃));
- (iv) it has the ability to regulate polarization of the plasma membrane;
- (v) it has the ability to modulate cell proliferation, cell migration, differentiation and/or cell survival;
- (vi) it has the ability to modulate function, survival, morphology, proliferation and/or differentiation of cells of tissues in which 65499 or 58875 molecules are expressed;
- (vii) it has a molecular weight (*e.g.*, deduced molecular weight), amino acid composition or other physical characteristic of a 65499 or 58875 protein of SEQ ID NO:2 or 4, respectively;

(viii) it has an overall sequence similarity (identity) of at least 60%, preferably at least 70%, more preferably at least 75, 78, 79, 80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99% or more, with a polypeptide of SEQ ID NO:2 or 4;

(ix) it has an N-terminal domain which is preferably about 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or higher, identical to a polypeptide of SEQ ID NO:2 or 4;

(x) it has at least one transmembrane domains which is preferably about 70%, 80%, 90%, 95% or higher, identical to a polypeptide of SEQ ID NO:2 or 4; or

(xi) it has a C-terminal domain which is preferably about 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or higher, identical to a polypeptide of SEQ ID NO:2 or 4.

10 In a preferred embodiment, the 65499 or 58875 protein, or fragment thereof, differs from the corresponding sequence in SEQ ID NO:2 or 4. In one embodiment it differs by at least one but by less than 15, 10 or 5 amino acid residues. In another it differs from the corresponding sequence in a polypeptide of SEQ ID NO:2 or 4 by at least one residue but less than 20%, 15%, 10% or 5% of the residues in it differ from the corresponding
15 sequence in a polypeptide of SEQ ID NO:2 or 4. (If this comparison requires alignment the sequences should be aligned for maximum homology. "Looped" out sequences from deletions or insertions, or mismatches, are considered differences.) The differences are, preferably, differences or changes at a non essential residue or a conservative substitution. In a preferred embodiment the differences are not in the seven transmembrane receptor
20 domain. In another embodiment one or more differences are in the seven transmembrane receptor domain.

Other embodiments include a protein that contain one or more changes in amino acid sequence, *e.g.*, a change in an amino acid residue which is not essential for activity. Such 65499 or 58875 proteins differ in amino acid sequence from SEQ ID NO:2 or 4, yet
25 retain biological activity.

In one embodiment, the protein includes an amino acid sequence at least about 60%, 65%, 70%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more homologous to a polypeptide of SEQ ID NO:2 or 4.

30 A 65499 or 58875 protein or fragment is provided which varies from the sequence of SEQ ID NO:2 or 4 by at least one but by less than 15, 10 or 5 amino acid residues in the protein or fragment but which does not differ from SEQ ID NO:2 or 4 in other regions. (If this comparison requires alignment the sequences should be aligned for maximum

homology. "Looped" out sequences from deletions or insertions, or mismatches, are considered differences.) In some embodiments the difference is at a non essential residue or is a conservative substitution, while in others the difference is at an essential residue or is a non conservative substitution.

5 In one embodiment, a biologically active portion of a 65499 or 58875 protein includes an N- or a C-terminal region of human 65499 or 58875. Alternatively, the biologically active portion of a 65499 or 58875 protein a transmembrane domain of human 65499 or 58875. Moreover, other biologically active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or
10 more of the functional activities of a native 65499 or 58875 protein.

In a preferred embodiment, the 65499 or 58875 protein has an amino acid sequence shown in SEQ ID NO:2 or 4, respectively. In other embodiments, the 65499 or 58875 protein is substantially identical to SEQ ID NO:2 or 4. In yet another embodiment, the 65499 or 58875 protein is substantially identical to SEQ ID NO:2 or 4 and retains the
15 functional activity of the protein of SEQ ID NO:2 or 4, as described above. Accordingly, in another embodiment, the 65499 or 58875 protein is a protein which includes an amino acid sequence at least about 60%, 65%, 70%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to SEQ ID NO:2 or 4.

20

65499 or 58875 Chimeric or Fusion Proteins

In another aspect, the invention provides 65499 or 58875 chimeric or fusion proteins. As used herein, a 65499 or 58875 "chimeric protein" or "fusion protein" includes a 65499 or 58875 polypeptide linked to a non-65499 or 58875 polypeptide. A "non-65499
25 or 58875 polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein which is not substantially homologous to the 65499 or 58875 protein, *e.g.*, a protein which is different from the 65499 or 58875 protein and which is derived from the same or a different organism. The 65499 or 58875 polypeptide of the fusion protein can correspond to all or a portion *e.g.*, a fragment described herein of a
30 65499 or 58875 amino acid sequence. In a preferred embodiment, a 65499 or 58875 fusion protein includes at least one (or two) biologically active portion of a 65499 or 58875 protein. The non-65499 or non-58875 polypeptide can be fused to the N-terminus or C-terminus of the 65499 or 58875 polypeptide.

The fusion protein can include a moiety which has a high affinity for a ligand. For example, the fusion protein can be a GST-65499 or -58875 fusion protein in which the 65499 or 58875 sequences are fused to the C-terminus of the GST sequences. Such fusion proteins can facilitate the purification of recombinant 65499 or 58875. Alternatively, the fusion protein can be a 65499 or 58875 protein containing a heterologous signal sequence at its N-terminus. In certain host cells (*e.g.*, mammalian host cells), expression and/or secretion of 65499 or 58875 can be increased through use of a heterologous signal sequence.

Fusion proteins can include all or a part of a serum protein, *e.g.*, a portion of an immunoglobulin (*e.g.*, IgG, IgA, or IgE), *e.g.*, an Fc region and/or the hinge C1 and C2 sequences of an immunoglobulin or human serum albumin.

The 65499 or 58875 fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject *in vivo*. The 65499 or 58875 fusion proteins can be used to affect the bioavailability of a 65499 or 58875 substrate. 65499 or 58875 fusion proteins may be useful therapeutically for the treatment of disorders caused by, for example, (i) aberrant modification or mutation of a gene encoding a 65499 or 58875 protein; (ii) mis-regulation of the 65499 or 58875 gene; and (iii) aberrant post-translational modification of a 65499 or 58875 protein.

Moreover, the 65499 or 58875-fusion proteins of the invention can be used as immunogens to produce anti-65499 or -58875 antibodies in a subject, to purify 65499 or 58875 ligands and in screening assays to identify molecules which inhibit the interaction of 65499 or 58875 with a 65499 or 58875 substrate.

Expression vectors are commercially available that already encode a fusion moiety (*e.g.*, a GST polypeptide). A 65499- or 58875-encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the 65499 or 58875 protein.

Variants of 65499 or 58875 Proteins

In another aspect, the invention also features a variant of a 65499 or 58875 polypeptide, *e.g.*, which functions as an agonist (mimetics) or as an antagonist. Variants of the 65499 or 58875 proteins can be generated by mutagenesis, *e.g.*, discrete point mutation, the insertion or deletion of sequences or the truncation of a 65499 or 58875 protein. An agonist of the 65499 or 58875 proteins can retain substantially the same, or a subset, of the

biological activities of the naturally occurring form of a 65499 or 58875 protein. An antagonist of a 65499 or 58875 protein can inhibit one or more of the activities of the naturally occurring form of the 65499 or 58875 protein by, for example, competitively modulating a 65499- or 58875-mediated activity of a 65499 or 58875 protein. Thus, specific biological effects can be elicited by treatment with a variant of limited function. Preferably, treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the protein has fewer side effects in a subject relative to treatment with the naturally occurring form of the 65499 or 58875 protein.

Variants of a 65499 or 58875 protein can be identified by screening combinatorial libraries of mutants, *e.g.*, truncation mutants, of a 65499 or 58875 protein for agonist or antagonist activity.

Libraries of fragments *e.g.*, N terminal, C terminal, or internal fragments, of a 65499 or 58875 protein coding sequence can be used to generate a variegated population of fragments for screening and subsequent selection of variants of a 65499 or 58875 protein.

Variants in which a cysteine residues is added or deleted or in which a residue which is glycosylated is added or deleted are particularly preferred.

Methods for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. Recursive ensemble mutagenesis (REM), a new technique which enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify 65499 or 58875 variants (Arkin and Yourvan (1992) *Proc. Natl. Acad. Sci. USA* 89:7811-7815; Delgrave *et al.* (1993) *Protein Engineering* 6(3):327-331).

Cell based assays can be exploited to analyze a variegated 65499 or 58875 library. For example, a library of expression vectors can be transfected into a cell line, *e.g.*, a cell line, which ordinarily responds to 65499 or 58875 in a substrate-dependent manner. The transfected cells are then contacted with 65499 or 58875 and the effect of the expression of the mutant on signaling by the 65499 or 58875 substrate can be detected, *e.g.*, by measuring changes in seven transmembrane receptor activity. Plasmid DNA can then be recovered from the cells which score for inhibition, or alternatively, potentiation of signaling by the 65499 or 58875 substrate, and the individual clones further characterized.

In another aspect, the invention features a method of making a 65499 or 58875 polypeptide, *e.g.*, a peptide having a non-wild type activity, *e.g.*, an antagonist, agonist, or

super agonist of a naturally occurring 65499 or 58875 polypeptide, *e.g.*, a naturally occurring 65499 or 58875 polypeptide. The method includes: altering the sequence of a 65499 or 58875 polypeptide, *e.g.*, altering the sequence, *e.g.*, by substitution or deletion of one or more residues of a non-conserved region, a domain or residue disclosed herein, and
5 testing the altered polypeptide for the desired activity.

In another aspect, the invention features a method of making a fragment or analog of a 65499 or 58875 polypeptide a biological activity of a naturally occurring 65499 or 58875 polypeptide. The method includes: altering the sequence, *e.g.*, by substitution or deletion of one or more residues, of a 65499 or 58875 polypeptide, *e.g.*, altering the
10 sequence of a non-conserved region, or a domain or residue described herein, and testing the altered polypeptide for the desired activity.

Anti-65499 or -58875 Antibodies

In another aspect, the invention provides an anti-65499 or -58875 antibody. The
15 term "antibody" as used herein refers to an immunoglobulin molecule or immunologically active portion thereof, *i.e.*, an antigen-binding portion. Examples of immunologically active fragments of immunoglobulin molecules include scFV and dcFV fragments, F(ab) and F(ab')₂ fragments which can be generated by treating the antibody with an enzyme such as papain or pepsin, respectively.

20 The antibody can be a polyclonal, monoclonal, recombinant, *e.g.*, a chimeric or humanized, fully human, non-human, *e.g.*, murine, or single chain antibody, or a fragment thereof. In a preferred embodiment, the antibody has effector function and can fix complement. The antibody or a fragment thereof can be coupled to a toxin or imaging agent.

25 A full-length 65499 or 58875 protein or, antigenic peptide fragment of 65499 or 58875 can be used as an immunogen or can be used to identify anti-65499 or -58875 antibodies made with other immunogens, *e.g.*, cells, membrane preparations, and the like. The antigenic peptide of 65499 or 58875 should include at least 8 amino acid residues of the amino acid sequence shown in SEQ ID NO:2 or 4 and encompasses an epitope of
30 65499 or 58875. Preferably, the antigenic peptide includes at least 10 amino acid residues, more preferably at least 15 amino acid residues, even more preferably at least 20 amino acid residues, and most preferably at least 30 amino acid residues.

Fragments of 65499 which include residues 98-105, 171-193 and/or 425-433 of SEQ ID NO:2 can be used to make, *e.g.*, used as immunogens or used to characterize the specificity of an antibody, antibodies against regions of the 65499 protein which are believed to be extracellular. Similarly, fragments of 65499 which include residues 32-56, 73-97, 106-127, 149-170, 194-218, 400-424 and/or 434-456 of SEQ ID NO: 2 can be used to make an antibody against a region of the 65499 protein which is believed to reside in the transmembrane. Fragments of 65499 which include residues 57-72, 128-148 and/or 219-399 of SEQ ID NO: 2 can be used to make an antibody against a region of the 65499 protein which is believed to be intracellular.

Fragments of 58875 which include residues 91-98, 172-198 and/or 271-287 of SEQ ID NO:4 can be used to make, *e.g.*, used as immunogens or used to characterize the specificity of an antibody, antibodies against regions of the 58875 protein which are believed to be extracellular. Similarly, fragments of 58875 which include residues 36-60, 70-90, 99-115, 153-171, 199-223, 252-270 and/or 288-309 of SEQ ID NO: 4 can be used to make an antibody against a region of the 58875 protein which is believed to reside in the transmembrane. Fragments of 58875 which include residues 61-69, 116-152 and/or 224-251 of SEQ ID NO: 4 can be used to make an antibody against a region of the 58875 protein which is believed to be intracellular.

Antibodies reactive with, or specific for, any of these regions, or other regions or domains described herein are provided.

Preferred epitopes encompassed by the antigenic peptide are regions of 65499 or 58875 are located on the surface of the protein, *e.g.*, hydrophilic regions, as well as regions with high antigenicity. For example, an Emimi surface probability analysis of the human 65499 or 58875 protein sequence can be used to indicate the regions that have a particularly high probability of being localized to the surface of the 65499 or 58875 protein and are thus likely to constitute surface residues useful for targeting antibody production.

In a preferred embodiment the antibody can bind to the extracellular portion of the 65499 or 58875 protein, *e.g.*, it can bind to a whole cell which expresses the 65499 or 58875 protein. In another embodiment, the antibody binds an intracellular portion of the 65499 or 58875 protein.

In a preferred embodiment the antibody binds an epitope on any domain or region on 65499 or 58875 proteins described herein.

Additionally, chimeric, humanized, and completely human antibodies are also within the scope of the invention. Chimeric, humanized, but most preferably, completely human antibodies are desirable for applications which include repeated administration, *e.g.*, therapeutic treatment (and some diagnostic applications) of human patients. Chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, can be made using standard recombinant DNA techniques. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in Robinson *et al.* International Application No. PCT/US86/02269; Akira, *et al.* European Patent Application 184,187; Taniguchi, M., European Patent Application 171,496; Morrison *et al.* European Patent Application 173,494; Neuberger *et al.* PCT International Publication No. WO 86/01533; Cabilly *et al.* U.S. Patent No. 4,816,567; Cabilly *et al.* European Patent Application 125,023; Better *et al.* (1988) *Science* 240:1041-1043; Liu *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:3439-3443; Liu *et al.* (1987) *J. Immunol.* 139:3521-3526; Sun *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:214-218; Nishimura *et al.* (1987) *Canc. Res.* 47:999-1005; Wood *et al.* (1985) *Nature* 314:446-449; and Shaw *et al.* (1988) *J. Natl. Cancer Inst.* 80:1553-1559; Morrison, S. L. (1985) *Science* 229:1202-1207; Oi *et al.* (1986) *BioTechniques* 4:214; Winter U.S. Patent 5,225,539; Jones *et al.* (1986) *Nature* 321:552-525; Verhoeven *et al.* (1988) *Science* 239:1534; and Beidler *et al.* (1988) *J. Immunol.* 141:4053-4060.

Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Such antibodies can be produced using transgenic mice that are incapable of expressing endogenous immunoglobulin heavy and light chains genes, but which can express human heavy and light chain genes. *See*, for example, Lonberg and Huszar (1995) *Int. Rev. Immunol.* 13:65-93; and U.S. Patent Nos. 5,625,126; 5,633,425; 5,569,825; 5,661,016; and 5,545,806. In addition, companies such as Abgenix, Inc. (Fremont, CA) and Medarex, Inc. (Princeton, NJ), can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

Completely human antibodies that recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, *e.g.*, a murine antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. This technology is described by Jespers *et al.* (1994) *Bio/Technology* 12:899-903).

The anti-65499 or 58875 antibody can be a single chain antibody. A single-chain antibody (scFV) can be engineered (see, for example, Colcher, D. *et al.* (1999) *Ann. NY Acad. Sci.* 880:263-80; and Reiter, Y. (1996) *Clin. Cancer Res.* 2:245-52). The single chain antibody can be dimerized or multimerized to generate multivalent antibodies having specificities for different epitopes of the same target 65499 or 58875 protein.

In a preferred embodiment, the antibody has reduced or no ability to bind an Fc receptor. For example, it is an isotype or subtype, fragment or other mutant, which does not support binding to an Fc receptor, *e.g.*, it has a mutagenized or deleted Fc receptor binding region. An antibody (or fragment thereof) may be conjugated to a therapeutic moiety such as a cytotoxin, a therapeutic agent or a radioactive metal ion. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.*, mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.*, daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.*, dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (*e.g.*, vincristine and vinblastine).

The conjugates of the invention can be used for modifying a given biological response, the drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, α -interferon, β -interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"),

granulocyte macrophase colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980.

5 An anti-65499 or 58875 antibody (*e.g.*, monoclonal antibody) can be used to isolate 65499 or 58875 by standard techniques, such as affinity chromatography or immunoprecipitation. Moreover, an anti-65499 or 58875 antibody can be used to detect 65499 or 58875 protein (*e.g.*, in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the protein. Anti-65499 or 58875 antibodies can be
10 used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, *e.g.*, to determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (*i.e.*, physically linking) the antibody to a detectable substance (*i.e.*, antibody labelling). Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive
15 materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a
20 luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I , ^{131}I , ^{35}S or ^3H .

 In preferred embodiments, an antibody can be made by immunizing with a purified 65499 or 58875 antigen, or a fragment thereof, *e.g.*, a fragment described herein, a
25 membrane associated antigen, tissues, *e.g.*, crude tissue preparations, whole cells, preferably living cells, lysed cells, or cell fractions, *e.g.*, membrane fractions.

 Antibodies which bind only a native 65499 or 58875 protein, only denatured or otherwise non-native 65499 or 58875 protein, or which bind both, are within the invention. Antibodies with linear or conformational epitopes are within the invention.
30 Conformational epitopes sometimes can be identified by identifying antibodies which bind to native but not denatured 65499 or 58875 protein.

Recombinant Expression Vectors, Host Cells and Genetically Engineered Cells

In another aspect, the invention includes, vectors, preferably expression vectors, containing a nucleic acid encoding a polypeptide described herein. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked and can include a plasmid, cosmid or viral vector. The vector can be capable of autonomous replication or it can integrate into a host DNA. Viral vectors include, *e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses.

A vector can include a 65499 or 58875 nucleic acid in a form suitable for expression of the nucleic acid in a host cell. Preferably the recombinant expression vector includes one or more regulatory sequences operatively linked to the nucleic acid sequence to be expressed. The term "regulatory sequence" includes promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Regulatory sequences include those which direct constitutive expression of a nucleotide sequence, as well as tissue-specific regulatory and/or inducible sequences. The design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, and the like. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or polypeptides, including fusion proteins or polypeptides, encoded by nucleic acids as described herein (*e.g.*, 65499 or 58875 proteins, mutant forms of 65499 or 58875 proteins, fusion proteins, and the like).

The recombinant expression vectors of the invention can be designed for expression of 65499 or 58875 proteins in prokaryotic or eukaryotic cells. For example, polypeptides of the invention can be expressed in *E. coli*, insect cells (*e.g.*, using baculovirus expression vectors), yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2) to increase the solubility of the recombinant protein; and 3) to aid in the purification of the

recombinant protein by acting as a ligand in affinity purification. Often, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith, D. B. and Johnson, K. S. (1988) *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Purified fusion proteins can be used in 65499 or 58875 activity assays, (e.g., direct assays or competitive assays described in detail below), or to generate antibodies specific for 65499 or 58875 proteins. In a preferred embodiment, a fusion protein expressed in a retroviral expression vector of the present invention can be used to infect bone marrow cells which are subsequently transplanted into irradiated recipients. The pathology of the subject recipient is then examined after sufficient time has passed (e.g., six (6) weeks).

To maximize recombinant protein expression in *E. coli* is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein (Gottesman, S., *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 119-128). Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (Wada *et al.*, (1992) *Nucleic Acids Res.* 20:2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

The 65499 or 58875 expression vector can be a yeast expression vector, a vector for expression in insect cells, e.g., a baculovirus expression vector or a vector suitable for expression in mammalian cells.

When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (e.g., tissue-specific regulatory elements are used to express the nucleic acid). Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific;

Pinkert *et al.* (1987) *Genes Dev.* 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) *Adv. Immunol.* 43:235-275), in particular promoters of T cell receptors (Winoto and Baltimore (1989) *EMBO J.* 8:729-733) and immunoglobulins (Banerji *et al.* (1983) *Cell* 33:729-740; Queen and Baltimore (1983) *Cell* 33:741-748), neuron-specific promoters (e.g., the neurofilament promoter; Byrne and Ruddle (1989) *Proc. Natl. Acad. Sci. USA* 86:5473-5477), pancreas-specific promoters (Edlund *et al.* (1985) *Science* 230:912-916), and mammary gland-specific promoters (e.g., milk whey promoter; U.S. Patent No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, for example, the murine hox promoters (Kessel and Gruss (1990) *Science* 249:374-379) and the α -fetoprotein promoter (Campes and Tilghman (1989) *Genes Dev.* 3:537-546).

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. Regulatory sequences (e.g., viral promoters and/or enhancers) operatively linked to a nucleic acid cloned in the antisense orientation can be chosen which direct the constitutive, tissue specific or cell type specific expression of antisense RNA in a variety of cell types. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus. For a discussion of the regulation of gene expression using antisense genes see Weintraub, H. *et al.*, Antisense RNA as a molecular tool for genetic analysis, *Reviews - Trends in Genetics*, Vol. 1(1) 1986.

Another aspect the invention provides a host cell which includes a nucleic acid molecule described herein, e.g., a 65499 or 58875 nucleic acid molecule within a recombinant expression vector or a 65499 or 58875 nucleic acid molecule containing sequences which allow it to homologously recombine into a specific site of the host cell's genome. The terms "host cell" and "recombinant host cell" are used interchangeably herein. Such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, a 65499 or 58875 protein can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or

mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into host cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (*e.g.*, DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation

A host cell of the invention can be used to produce (*i.e.*, express) a 65499 or 58875 protein. Accordingly, the invention further provides methods for producing a 65499 or 58875 protein using the host cells of the invention. In one embodiment, the method includes culturing the host cell of the invention (into which a recombinant expression vector encoding a 65499 or 58875 protein has been introduced) in a suitable medium such that a 65499 or 58875 protein is produced. In another embodiment, the method further includes isolating a 65499 or 58875 protein from the medium or the host cell.

In another aspect, the invention features, a cell or purified preparation of cells which include a 65499 or 58875 transgene, or which otherwise misexpress 65499 or 58875. The cell preparation can consist of human or non human cells, *e.g.*, rodent cells, *e.g.*, mouse or rat cells, rabbit cells, or pig cells. In preferred embodiments, the cell or cells include a 65499 or 58875 transgene, *e.g.*, a heterologous form of a 65499 or 58875, *e.g.*, a gene derived from humans (in the case of a non-human cell). The 65499 or 58875 transgene can be misexpressed, *e.g.*, overexpressed or underexpressed. In other preferred embodiments, the cell or cells include a gene which misexpress an endogenous 65499 or 58875, *e.g.*, a gene the expression of which is disrupted, *e.g.*, a knockout. Such cells can serve as a model for studying disorders which are related to mutated or mis-expressed 65499 or 58875 alleles or for use in drug screening.

In another aspect, the invention features, a human cell, *e.g.*, a hematopoietic stem cell, transformed with nucleic acid which encodes a subject 65499 or 58875 polypeptide.

Also provided are cells, *e.g.*, human cells, *e.g.*, human hematopoietic or fibroblast cells in which an endogenous 65499 or 58875 is under the control of a regulatory sequence that does not normally control the expression of the endogenous 65499 or 58875 gene. The expression characteristics of an endogenous gene within a cell, *e.g.*, a cell line or microorganism, can be modified by inserting a heterologous DNA regulatory element into the genome of the cell such that the inserted regulatory element is operably linked to the

endogenous 65499 or 58875 gene. For example, an endogenous 65499 or 58875 gene, *e.g.*, a gene which is “transcriptionally silent,” *e.g.*, not normally expressed, or expressed only at very low levels, may be activated by inserting a regulatory element which is capable of promoting the expression of a normally expressed gene product in that cell. Techniques
5 such as targeted homologous recombinations, can be used to insert the heterologous DNA as described in, *e.g.*, Chappel, US 5,272,071; WO 91/06667, published in May 16, 1991.

Transgenic Animals

The invention provides non-human transgenic animals. Such animals are useful for
10 studying the function and/or activity of a 65499 or 58875 protein and for identifying and/or evaluating modulators of 65499 or 58875 activity. As used herein, a “transgenic animal” is a non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats,
15 chickens, amphibians, and the like. A transgene is exogenous DNA or a rearrangement, *e.g.*, a deletion of endogenous chromosomal DNA, which preferably is integrated into or occurs in the genome of the cells of a transgenic animal. A transgene can direct the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal, other transgenes, *e.g.*, a knockout, reduce expression. Thus, a transgenic animal
20 can be one in which an endogenous 65499 or 58875 gene has been altered by, *e.g.*, by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, *e.g.*, an embryonic cell of the animal, prior to development of the animal.

Intronic sequences and polyadenylation signals can also be included in the
25 transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably linked to a transgene of the invention to direct expression of a 65499 or 58875 protein to particular cells. A transgenic founder animal can be identified based upon the presence of a 65499 or 58875 transgene in its genome and/or expression of 65499 or 58875 mRNA in tissues or cells of the animals. A transgenic
30 founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene encoding a 65499 or 58875 protein can further be bred to other transgenic animals carrying other transgenes.

65499 or 58875 proteins or polypeptides can be expressed in transgenic animals or plants, *e.g.*, a nucleic acid encoding the protein or polypeptide can be introduced into the genome of an animal. In preferred embodiments the nucleic acid is placed under the control of a tissue specific promoter, *e.g.*, a milk or egg specific promoter, and recovered
5 from the milk or eggs produced by the animal. Suitable animals are mice, pigs, cows, goats, and sheep.

The invention also includes a population of cells from a transgenic animal, as discussed, *e.g.*, below.

10 Uses

The nucleic acid molecules, proteins, protein homologues, and antibodies described herein can be used in one or more of the following methods: a) screening assays; b) predictive medicine (*e.g.*, diagnostic assays, prognostic assays, monitoring clinical trials, and pharmacogenetics); and c) methods of treatment (*e.g.*, therapeutic and prophylactic).

15 The isolated nucleic acid molecules of the invention can be used, for example, to express a 65499 or 58875 protein (*e.g.*, via a recombinant expression vector in a host cell in gene therapy applications), to detect a 65499 or 58875 mRNA (*e.g.*, in a biological sample) or a genetic alteration in a 65499 or 58875 gene, and to modulate 65499 or 58875 activity, as described further below. The 65499 or 58875 proteins can be used to treat disorders
20 characterized by insufficient or excessive production of a 65499 or 58875 substrate or production of 65499 or 58875 inhibitors. In addition, the 65499 or 58875 proteins can be used to screen for naturally occurring 65499 or 58875 substrates, to screen for drugs or compounds which modulate 65499 or 58875 activity, as well as to treat disorders characterized by insufficient or excessive production of 65499 or 58875 protein or
25 production of 65499 or 58875 protein forms which have decreased, aberrant or unwanted activity compared to 65499 or 58875 wild type protein. Exemplary disorders include: conditions involving aberrant or deficient transmission of an extracellular signal into a cell, for example, a bone cell (*e.g.*, an osteoclast or an osteoblast), a hematopoietic cell, a neural cell, a heart cell); conditions involving aberrant or deficient mobilization of an intracellular
30 molecule that participates in a signal transduction pathway; and/or conditions involving aberrant or deficient modulation of function, survival, morphology, proliferation and/or differentiation of cells of tissues in which 65499 or 58875 molecules are expressed.

Moreover, the anti-65499 or 58875 antibodies of the invention can be used to detect and

isolate 65499 or 58875 proteins, regulate the bioavailability of 65499 or 58875 proteins, and modulate 65499 or 58875 activity.

A method of evaluating a compound for the ability to interact with, *e.g.*, bind, a subject 65499 or 58875 polypeptide is provided. The method includes: contacting the compound with the subject 65499 or 58875 polypeptide; and evaluating ability of the compound to interact with, *e.g.*, to bind or form a complex with the subject 65499 or 58875 polypeptide. This method can be performed *in vitro*, *e.g.*, in a cell free system, or *in vivo*, *e.g.*, in a two-hybrid interaction trap assay. This method can be used to identify naturally occurring molecules which interact with subject 65499 or 58875 polypeptide. It can also be used to find natural or synthetic inhibitors of subject 65499 or 58875 polypeptide. Screening methods are discussed in more detail below.

Screening Assays

The invention provides methods (also referred to herein as “screening assays”) for identifying modulators, *i.e.*, candidate or test compounds or agents (*e.g.*, proteins, peptides, peptidomimetics, peptoids, small molecules or other drugs) which bind to 65499 or 58875 proteins, have a stimulatory or inhibitory effect on, for example, 65499 or 58875 expression or 65499 or 58875 activity, or have a stimulatory or inhibitory effect on, for example, the expression or activity of a 65499 or 58875 substrate. Compounds thus identified can be used to modulate the activity of target gene products (*e.g.*, 65499 or 58875 genes) in a therapeutic protocol, to elaborate the biological function of the target gene product, or to identify compounds that disrupt normal target gene interactions.

In one embodiment, the invention provides assays for screening candidate or test compounds which are substrates of a 65499 or 58875 protein or polypeptide or a biologically active portion thereof. In another embodiment, the invention provides assays for screening candidate or test compounds which bind to or modulate the activity of a 65499 or 58875 protein or polypeptide or a biologically active portion thereof.

In any screening assay, a 65499 or 58875 polypeptide which may have an extracellular region, or an intracellular region can be used.

The test compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; peptoid libraries [libraries of molecules having the functionalities of peptides, but with a novel, non-peptide backbone which are resistant to enzymatic

degradation but which nevertheless remain bioactive] (see, e.g., Zuckermann, R.N. *et al. J. Med. Chem.* 1994, 37: 2678-85); spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection.

- 5 The biological library and peptoid library approaches are limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, K. S. (1997) *Anticancer Drug Des.* 12:145).

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt *et al.* (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:6909; Erb *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:11422; Zuckermann *et al.* (1994). *J. Med. Chem.* 37:2678; Cho *et al.* (1993) *Science* 261:1303; Carrell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2059; Carrell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2061; and in Gallop *et al.* (1994) *J. Med. Chem.* 37:1233.

Libraries of compounds may be presented in solution (e.g., Houghten (1992) *Biotechniques* 13:412-421), or on beads (Lam (1991) *Nature* 354:82-84), chips (Fodor (1993) *Nature* 364:555-556), bacteria (Ladner USP 5,223,409), spores (Ladner USP '409), plasmids (Cull *et al.* (1992) *Proc Natl Acad Sci USA* 89:1865-1869) or on phage (Scott and Smith (1990) *Science* 249:386-390); (Devlin (1990) *Science* 249:404-406); (Cwirla *et al.* (1990) *Proc. Natl. Acad. Sci.* 87:6378-6382); (Felici (1991) *J. Mol. Biol.* 222:301-310); (Ladner *supra.*).

In one embodiment, an assay is a cell-based assay in which a cell which expresses a 65499 or 58875 protein or biologically active portion thereof is contacted with a test compound, and the ability of the test compound to modulate 65499 or 58875 activity is determined. Determining the ability of the test compound to modulate 65499 or 58875 activity can be accomplished by monitoring, for example, changes in seven transmembrane receptor activity. The cell, for example, can be of mammalian origin.

The ability of the test compound to modulate 65499 or 58875 binding to a compound, e.g., a 65499 or 58875 substrate, or to bind to 65499 or 58875 can also be evaluated. This can be accomplished, for example, by coupling the compound, e.g., the substrate, with a radioisotope or enzymatic label such that binding of the compound, e.g., the substrate, to 65499 or 58875 can be determined by detecting the labeled compound, e.g., substrate, in a complex. Alternatively, 65499 or 58875 could be coupled with a radioisotope or enzymatic label to monitor the ability of a test compound to modulate

65499 or 58875 binding to a 65499 or 58875 substrate in a complex. For example, compounds (*e.g.*, 65499 or 58875 substrates) can be labeled with ^{125}I , ^{35}S , ^{14}C , or ^3H , either directly or indirectly, and the radioisotope detected by direct counting of radioemmission or by scintillation counting. Alternatively, compounds can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product.

The ability of a compound (*e.g.*, a 65499 or 58875 substrate) to interact with 65499 or 58875 with or without the labeling of any of the interactants can be evaluated. For example, a microphysiometer can be used to detect the interaction of a compound with 65499 or 58875 without the labeling of either the compound or the 65499 or 58875. McConnell, H. M. *et al.* (1992) *Science* 257:1906-1912. As used herein, a “microphysiometer” (*e.g.*, Cytosensor) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between a compound and 65499 or 58875.

In yet another embodiment, a cell-free assay is provided in which a 65499 or 58875 protein or biologically active portion thereof is contacted with a test compound and the ability of the test compound to bind to the 65499 or 58875 protein or biologically active portion thereof is evaluated. Preferred biologically active portions of the 65499 or 58875 proteins to be used in assays of the present invention include fragments which participate in interactions with non-65499 or 58875 molecules, *e.g.*, fragments with high surface probability scores.

Soluble and/or membrane-bound forms of isolated proteins (*e.g.*, 65499 or 58875 proteins or biologically active portions thereof) can be used in the cell-free assays of the invention. When membrane-bound forms of the protein are used, it may be desirable to utilize a solubilizing agent. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-dodecylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton[®] X-100, Triton[®] X-114, Thesit[®], Isotridecypoly(ethylene glycol ether)_n, 3-[(3-cholamidopropyl)dimethylamminio]-1-propane sulfonate (CHAPS), 3-[(3-cholamidopropyl)dimethylamminio]-2-hydroxy-1-

propane sulfonate (CHAPSO), or N-dodecyl=N,N-dimethyl-3-ammonio-1-propane sulfonate.

Cell-free assays involve preparing a reaction mixture of the target gene protein and the test compound under conditions and for a time sufficient to allow the two components
5 to interact and bind, thus forming a complex that can be removed and/or detected.

The interaction between two molecules can also be detected, *e.g.*, using fluorescence energy transfer (FET) (see, for example, Lakowicz *et al.*, U.S. Patent No. 5,631,169; Stavrianopoulos, *et al.*, U.S. Patent No. 4,868,103). A fluorophore label on the first, 'donor' molecule is selected such that its emitted fluorescent energy will be absorbed
10 by a fluorescent label on a second, 'acceptor' molecule, which in turn is able to fluoresce due to the absorbed energy. Alternately, the 'donor' protein molecule may simply utilize the natural fluorescent energy of tryptophan residues. Labels are chosen that emit different wavelengths of light, such that the 'acceptor' molecule label may be differentiated from that of the 'donor'. Since the efficiency of energy transfer between the labels is related to
15 the distance separating the molecules, the spatial relationship between the molecules can be assessed. In a situation in which binding occurs between the molecules, the fluorescent emission of the 'acceptor' molecule label in the assay should be maximal. An FET binding event can be conveniently measured through standard fluorometric detection means well known in the art (*e.g.*, using a fluorimeter).

In another embodiment, determining the ability of the 65499 or 58875 protein to
20 bind to a target molecule can be accomplished using real-time Biomolecular Interaction Analysis (BIA) (see, *e.g.*, Sjolander, S. and Urbaniczky, C. (1991) *Anal. Chem.* 63:2338-2345 and Szabo *et al.* (1995) *Curr. Opin. Struct. Biol.* 5:699-705). "Surface plasmon resonance" or "BIA" detects biospecific interactions in real time, without labeling any of
25 the interactants (*e.g.*, BIAcore). Changes in the mass at the binding surface (indicative of a binding event) result in alterations of the refractive index of light near the surface (the optical phenomenon of surface plasmon resonance (SPR)), resulting in a detectable signal which can be used as an indication of real-time reactions between biological molecules.

In one embodiment, the target gene product or the test substance is anchored onto a
30 solid phase. The target gene product/test compound complexes anchored on the solid phase can be detected at the end of the reaction. Preferably, the target gene product can be anchored onto a solid surface, and the test compound, (which is not anchored), can be labeled, either directly or indirectly, with detectable labels discussed herein.

It may be desirable to immobilize either 65499 or 58875, an anti 65499 or 58875 antibody or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to a 65499 or 58875 protein, or interaction of a 65499 or 58875 protein with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows one or both of the proteins to be bound to a matrix. For example, glutathione-S-transferase/65499 or 58875 fusion proteins or glutathione-S-transferase/target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtiter plates, which are then combined with the test compound or the test compound and either the non-adsorbed target protein or 65499 or 58875 protein, and the mixture incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of 65499 or 58875 binding or activity determined using standard techniques.

Other techniques for immobilizing either a 65499 or 58875 protein or a target molecule on matrices include using conjugation of biotin and streptavidin. Biotinylated 65499 or 58875 protein or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques known in the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, IL), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical).

In order to conduct the assay, the non-immobilized component is added to the coated surface containing the anchored component. After the reaction is complete, unreacted components are removed (*e.g.*, by washing) under conditions such that any complexes formed will remain immobilized on the solid surface. The detection of complexes anchored on the solid surface can be accomplished in a number of ways. Where the previously non-immobilized component is pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. Where the previously non-immobilized component is not pre-labeled, an indirect label can be used to detect

complexes anchored on the surface; *e.g.*, using a labeled antibody specific for the immobilized component (the antibody, in turn, can be directly labeled or indirectly labeled with, *e.g.*, a labeled anti-Ig antibody).

In one embodiment, this assay is performed utilizing antibodies reactive with 65499 or 58875 protein or target molecules but which do not interfere with binding of the 65499 or 58875 protein to its target molecule. Such antibodies can be derivatized to the wells of the plate, and unbound target or 65499 or 58875 protein trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the 65499 or 58875 protein or target molecule, as well as enzyme-linked assays which rely on detecting an enzymatic activity associated with the 65499 or 58875 protein or target molecule.

Alternatively, cell free assays can be conducted in a liquid phase. In such an assay, the reaction products are separated from unreacted components, by any of a number of standard techniques, including but not limited to: differential centrifugation (see, for example, Rivas, G. and Minton, A.P., *Trends Biochem Sci* 1993 Aug;18(8):284-7); chromatography (gel filtration chromatography, ion-exchange chromatography); electrophoresis (see, *e.g.*, Ausubel, F. *et al.*, eds. *Current Protocols in Molecular Biology* 1999, J. Wiley: New York.); and immunoprecipitation (see, for example, Ausubel, F. *et al.*, eds. *Current Protocols in Molecular Biology* 1999, J. Wiley: New York). Such resins and chromatographic techniques are known to one skilled in the art (see, *e.g.*, Heegaard, N. H., *J Mol Recognit* 1998 Winter;11(1-6):141-8; Hage, D.S., and Tweed, S. A. *J Chromatogr B Biomed Sci Appl* 1997 Oct 10;699(1-2):499-525). Further, fluorescence energy transfer may also be conveniently utilized, as described herein, to detect binding without further purification of the complex from solution.

In a preferred embodiment, the assay includes contacting the 65499 or 58875 protein or biologically active portion thereof with a known compound which binds 65499 or 58875 to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a 65499 or 58875 protein, wherein determining the ability of the test compound to interact with a 65499 or 58875 protein includes determining the ability of the test compound to preferentially bind to 65499 or 58875 or biologically active portion thereof, or to modulate the activity of a target molecule, as compared to the known compound.

The target gene products of the invention can, *in vivo*, interact with one or more cellular or extracellular macromolecules, such as proteins. For the purposes of this discussion, such cellular and extracellular macromolecules are referred to herein as "binding partners." Compounds that disrupt such interactions can be useful in regulating the activity of the target gene product. Such compounds can include, but are not limited to molecules such as antibodies, peptides, and small molecules. The preferred target genes/products for use in this embodiment are the 65499 or 58875 genes herein identified. In an alternative embodiment, the invention provides methods for determining the ability of the test compound to modulate the activity of a 65499 or 58875 protein through modulation of the activity of a downstream effector of a 65499 or 58875 target molecule. For example, the activity of the effector molecule on an appropriate target can be determined, or the binding of the effector to an appropriate target can be determined, as previously described.

To identify compounds that interfere with the interaction between the target gene product and its cellular or extracellular binding partner(s), a reaction mixture containing the target gene product and the binding partner is prepared, under conditions and for a time sufficient, to allow the two products to form complex. In order to test an inhibitory agent, the reaction mixture is provided in the presence and absence of the test compound. The test compound can be initially included in the reaction mixture, or can be added at a time subsequent to the addition of the target gene and its cellular or extracellular binding partner. Control reaction mixtures are incubated without the test compound or with a placebo. The formation of any complexes between the target gene product and the cellular or extracellular binding partner is then detected. The formation of a complex in the control reaction, but not in the reaction mixture containing the test compound, indicates that the compound interferes with the interaction of the target gene product and the interactive binding partner. Additionally, complex formation within reaction mixtures containing the test compound and normal target gene product can also be compared to complex formation within reaction mixtures containing the test compound and mutant target gene product. This comparison can be important in those cases wherein it is desirable to identify compounds that disrupt interactions of mutant but not normal target gene products.

These assays can be conducted in a heterogeneous or homogeneous format. Heterogeneous assays involve anchoring either the target gene product or the binding partner onto a solid phase, and detecting complexes anchored on the solid phase at the end of the reaction. In homogeneous assays, the entire reaction is carried out in a liquid phase.

In either approach, the order of addition of reactants can be varied to obtain different information about the compounds being tested. For example, test compounds that interfere with the interaction between the target gene products and the binding partners, *e.g.*, by competition, can be identified by conducting the reaction in the presence of the test substance. Alternatively, test compounds that disrupt preformed complexes, *e.g.*, compounds with higher binding constants that displace one of the components from the complex, can be tested by adding the test compound to the reaction mixture after complexes have been formed. The various formats are briefly described below.

10 In a heterogeneous assay system, either the target gene product or the interactive cellular or extracellular binding partner, is anchored onto a solid surface (*e.g.*, a microtiter plate), while the non-anchored species is labeled, either directly or indirectly. The anchored species can be immobilized by non-covalent or covalent attachments. Alternatively, an immobilized antibody specific for the species to be anchored can be used to anchor the species to the solid surface.

15 In order to conduct the assay, the partner of the immobilized species is exposed to the coated surface with or without the test compound. After the reaction is complete, unreacted components are removed (*e.g.*, by washing) and any complexes formed will remain immobilized on the solid surface. Where the non-immobilized species is pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. Where the non-immobilized species is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; *e.g.*, using a labeled antibody specific for the initially non-immobilized species (the antibody, in turn, can be directly labeled or indirectly labeled with, *e.g.*, a labeled anti-Ig antibody). Depending upon the order of addition of reaction components, test compounds that inhibit complex formation or that
20
25 disrupt preformed complexes can be detected.

Alternatively, the reaction can be conducted in a liquid phase in the presence or absence of the test compound, the reaction products separated from unreacted components, and complexes detected; *e.g.*, using an immobilized antibody specific for one of the binding components to anchor any complexes formed in solution, and a labeled antibody specific
30 for the other partner to detect anchored complexes. Again, depending upon the order of addition of reactants to the liquid phase, test compounds that inhibit complex or that disrupt preformed complexes can be identified.

In an alternate embodiment of the invention, a homogeneous assay can be used. For example, a preformed complex of the target gene product and the interactive cellular or extracellular binding partner product is prepared in that either the target gene products or their binding partners are labeled, but the signal generated by the label is quenched due to complex formation (see, *e.g.*, U.S. Patent No. 4,109,496 that utilizes this approach for immunoassays). The addition of a test substance that competes with and displaces one of the species from the preformed complex will result in the generation of a signal above background. In this way, test substances that disrupt target gene product-binding partner interaction can be identified.

In yet another aspect, the 65499 or 58875 proteins can be used as "bait proteins" in a two-hybrid assay or three-hybrid assay (see, *e.g.*, U.S. Patent No. 5,283,317; Zervos *et al.* (1993) *Cell* 72:223-232; Madura *et al.* (1993) *J. Biol. Chem.* 268:12046-12054; Bartel *et al.* (1993) *Biotechniques* 14:920-924; Iwabuchi *et al.* (1993) *Oncogene* 8:1693-1696; and Brent WO94/10300), to identify other proteins, which bind to or interact with 65499 or 58875 ("65499 or 58875-binding proteins" or "65499 or 58875-bp") and are involved in 65499 or 58875 activity. Such 65499 or 58875-bps can be activators or inhibitors of signals by the 65499 or 58875 proteins or 65499 or 58875 targets as, for example, downstream elements of a 65499 or 58875-mediated signaling pathway.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for a 65499 or 58875 protein is fused to a gene encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. (Alternatively the: 65499 or 58875 protein can be the fused to the activator domain.) If the "bait" and the "prey" proteins are able to interact, *in vivo*, forming a 65499 or 58875-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, LacZ) which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene which encodes the protein which interacts with the 65499 or 58875 protein.

In another embodiment, modulators of 65499 or 58875 expression are identified. For example, a cell or cell free mixture is contacted with a candidate compound and the expression of 65499 or 58875 mRNA or protein evaluated relative to the level of expression of 65499 or 58875 mRNA or protein in the absence of the candidate compound.

5 When expression of 65499 or 58875 mRNA or protein is greater in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of 65499 or 58875 mRNA or protein expression. Alternatively, when expression of 65499 or 58875 mRNA or protein is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an

10 inhibitor of 65499 or 58875 mRNA or protein expression. The level of 65499 or 58875 mRNA or protein expression can be determined by methods described herein for detecting 65499 or 58875 mRNA or protein.

In another aspect, the invention pertains to a combination of two or more of the assays described herein. For example, a modulating agent can be identified using a cell-

15 based or a cell free assay, and the ability of the agent to modulate the activity of a 65499 or 58875 protein can be confirmed *in vivo*, e.g., in an animal such as an animal model for a GPCR-disease.

This invention further pertains to novel agents identified by the above-described screening assays. Accordingly, it is within the scope of this invention to further use an

20 agent identified as described herein (e.g., a 65499 or 58875 modulating agent, an antisense 65499 or 58875 nucleic acid molecule, a 65499 or 58875-specific antibody, or a 65499 or 58875-binding partner) in an appropriate animal model to determine the efficacy, toxicity, side effects, or mechanism of action, of treatment with such an agent. Furthermore, novel agents identified by the above-described screening assays can be used for treatments as

25 described herein.

Detection Assays

Portions or fragments of the nucleic acid sequences identified herein can be used as polynucleotide reagents. For example, these sequences can be used to: (i) map their

30 respective genes on a chromosome e.g., to locate gene regions associated with genetic disease or to associate 65499 or 58875 with a disease; (ii) identify an individual from a minute biological sample (tissue typing); and (iii) aid in forensic identification of a biological sample. These applications are described in the subsections below.

Chromosome Mapping

The 65499 or 58875 nucleotide sequences or portions thereof can be used to map the location of the 65499 or 58875 genes on a chromosome. This process is called
5 chromosome mapping. Chromosome mapping is useful in correlating the 65499 or 58875 sequences with genes associated with disease.

Briefly, 65499 or 58875 genes can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp in length) from the 65499 or 58875 nucleotide sequences. These primers can then be used for PCR screening of somatic cell hybrids containing
10 individual human chromosomes. Only those hybrids containing the human gene corresponding to the 65499 or 58875 sequences will yield an amplified fragment.

A panel of somatic cell hybrids in which each cell line contains either a single human chromosome or a small number of human chromosomes, and a full set of mouse chromosomes, can allow easy mapping of individual genes to specific human
15 chromosomes. (D'Eustachio, P. *et al.* (1983) *Science* 220:919-924).

Other mapping strategies *e.g.*, *in situ* hybridization (described in Fan, Y. *et al.* (1990) *Proc. Natl. Acad. Sci. USA*, 87:6223-27), pre-screening with labeled flow-sorted chromosomes, and pre-selection by hybridization to chromosome specific cDNA libraries can be used to map 65499 or 58875 to a chromosomal location.

20 Fluorescence *in situ* hybridization (FISH) of a DNA sequence to a metaphase chromosomal spread can further be used to provide a precise chromosomal location in one step. The FISH technique can be used with a DNA sequence as short as 500 or 600 bases. However, clones larger than 1,000 bases have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. Preferably
25 1,000 bases, and more preferably 2,000 bases will suffice to get good results at a reasonable amount of time. For a review of this technique, see Verma *et al.*, *Human Chromosomes: A Manual of Basic Techniques* (Pergamon Press, New York 1988).

Reagents for chromosome mapping can be used individually to mark a single chromosome or a single site on that chromosome, or panels of reagents can be used for
30 marking multiple sites and/or multiple chromosomes. Reagents corresponding to noncoding regions of the genes actually are preferred for mapping purposes. Coding sequences are more likely to be conserved within gene families, thus increasing the chance of cross hybridizations during chromosomal mapping.

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. (Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man, available on-line through Johns Hopkins University Welch Medical Library). The relationship between a gene and a disease, mapped to the same chromosomal region, can then be identified through linkage analysis (co-inheritance of physically adjacent genes), described in, for example, Egeland, J. *et al.* (1987) *Nature*, 325:783-787.

Moreover, differences in the DNA sequences between individuals affected and unaffected with a disease associated with the 65499 or 58875 gene, can be determined. If a mutation is observed in some or all of the affected individuals but not in any unaffected individuals, then the mutation is likely to be the causative agent of the particular disease. Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes, such as deletions or translocations that are visible from chromosome spreads or detectable using PCR based on that DNA sequence. Ultimately, complete sequencing of genes from several individuals can be performed to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

Tissue Typing

65499 or 58875 sequences can be used to identify individuals from biological samples using, *e.g.*, restriction fragment length polymorphism (RFLP). In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, the fragments separated, *e.g.*, in a Southern blot, and probed to yield bands for identification. The sequences of the present invention are useful as additional DNA markers for RFLP (described in U.S. Patent 5,272,057).

Furthermore, the sequences of the present invention can also be used to determine the actual base-by-base DNA sequence of selected portions of an individual's genome. Thus, the 65499 or 58875 nucleotide sequences described herein can be used to prepare two PCR primers from the 5' and 3' ends of the sequences. These primers can then be used to amplify an individual's DNA and subsequently sequence it. Panels of corresponding DNA sequences from individuals, prepared in this manner, can provide unique individual identifications, as each individual will have a unique set of such DNA sequences due to allelic differences.

Allelic variation occurs to some degree in the coding regions of these sequences, and to a greater degree in the noncoding regions. Each of the sequences described herein can, to some degree, be used as a standard against which DNA from an individual can be compared for identification purposes. Because greater numbers of polymorphisms occur in the noncoding regions, fewer sequences are necessary to differentiate individuals. The noncoding sequences of SEQ ID NO:1 can provide positive individual identification with a panel of perhaps 10 to 1,000 primers which each yield a noncoding amplified sequence of 100 bases. If predicted coding sequences, such as those in SEQ ID NO:3 are used, a more appropriate number of primers for positive individual identification would be 500-2,000.

If a panel of reagents from 65499 or 58875 nucleotide sequences described herein is used to generate a unique identification database for an individual, those same reagents can later be used to identify tissue from that individual. Using the unique identification database, positive identification of the individual, living or dead, can be made from extremely small tissue samples.

Use of Partial 65499 or 58875 Sequences in Forensic Biology

DNA-based identification techniques can also be used in forensic biology. To make such an identification, PCR technology can be used to amplify DNA sequences taken from very small biological samples such as tissues, *e.g.*, hair or skin, or body fluids, *e.g.*, blood, saliva, or semen found at a crime scene. The amplified sequence can then be compared to a standard, thereby allowing identification of the origin of the biological sample.

The sequences of the present invention can be used to provide polynucleotide reagents, *e.g.*, PCR primers, targeted to specific loci in the human genome, which can enhance the reliability of DNA-based forensic identifications by, for example, providing another "identification marker" (*i.e.* another DNA sequence that is unique to a particular individual). As mentioned above, actual base sequence information can be used for identification as an accurate alternative to patterns formed by restriction enzyme generated fragments. Sequences targeted to noncoding regions of SEQ ID NO:1 (*e.g.*, fragments derived from the noncoding regions of SEQ ID NO:1 having a length of at least 20 bases, preferably at least 30 bases) are particularly appropriate for this use.

The 65499 or 58875 nucleotide sequences described herein can further be used to provide polynucleotide reagents, *e.g.*, labeled or labelable probes which can be used in, for example, an *in situ* hybridization technique, to identify a specific tissue, *e.g.*, a tissue

containing bone cells. This can be very useful in cases where a forensic pathologist is presented with a tissue of unknown origin. Panels of such 65499 or 58875 probes can be used to identify tissue by species and/or by organ type.

In a similar fashion, these reagents, *e.g.*, 65499 or 58875 primers or probes can be used to screen tissue culture for contamination (*i.e.* screen for the presence of a mixture of different types of cells in a culture).

Predictive Medicine

The present invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual.

Generally, the invention provides, a method of determining if a subject is at risk for a disorder related to a lesion in or the misexpression of a gene which encodes a 65499 or 58875 polypeptide.

Such disorders include, *e.g.*, a disorder associated with the misexpression of a 65499 or 58875 polypeptide; cellular proliferative and/or differentiative disorders, disorders associated with bone metabolism, hematopoietic disorders, cardiovascular disorders, including endothelial cell disorders, brain disorders, a neurodegenerative disorder, and hormonal disorders.

The method includes one or more of the following:

detecting, in a tissue of the subject, the presence or absence of a mutation which affects the expression of the 65499 or 58875 gene, or detecting the presence or absence of a mutation in a region which controls the expression of the gene, *e.g.*, a mutation in the 5' control region;

detecting, in a tissue of the subject, the presence or absence of a mutation which alters the structure of the 65499 or 58875 gene;

detecting, in a tissue of the subject, the misexpression of the 65499 or 58875 gene, at the mRNA level, *e.g.*, detecting a non-wild type level of a mRNA ;

detecting, in a tissue of the subject, the misexpression of the gene, at the protein level, *e.g.*, detecting a non-wild type level of a 65499 or 58875 polypeptide.

In preferred embodiments the method includes: ascertaining the existence of at least one of: a deletion of one or more nucleotides from the 65499 or 58875 gene; an insertion of one or more nucleotides into the gene, a point mutation, *e.g.*, a substitution of one or more

nucleotides of the gene, a gross chromosomal rearrangement of the gene, *e.g.*, a translocation, inversion, or deletion.

For example, detecting the genetic lesion can include: (i) providing a probe/primer including an oligonucleotide containing a region of nucleotide sequence which hybridizes to a sense or antisense sequence from SEQ ID NO:1, or naturally occurring mutants thereof or 5' or 3' flanking sequences naturally associated with the 65499 or 58875 gene; (ii) exposing the probe/primer to nucleic acid of the tissue; and detecting, by hybridization, *e.g.*, *in situ* hybridization, of the probe/primer to the nucleic acid, the presence or absence of the genetic lesion.

In preferred embodiments detecting the misexpression includes ascertaining the existence of at least one of: an alteration in the level of a messenger RNA transcript of the 65499 or 58875 gene; the presence of a non-wild type splicing pattern of a messenger RNA transcript of the gene; or a non-wild type level of the 65499 or 58875 gene.

Methods of the invention can be used for prenatal screening, or to determine if a subject's offspring will be at risk for a disorder.

In preferred embodiments the method includes determining the structure of a 65499 or 58875 gene, an abnormal structure being indicative of risk for the disorder.

In preferred embodiments the method includes contacting a sample from the subject with an antibody to the 65499 or 58875 protein or a nucleic acid, which hybridizes specifically with the gene. These and other embodiments are discussed below.

Diagnostic and Prognostic Assays

The presence, level, or absence of 65499 or 58875 protein or nucleic acid in a biological sample can be evaluated by obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting 65499 or 58875 protein or nucleic acid (*e.g.*, mRNA, genomic DNA) that encodes 65499 or 58875 protein such that the presence of 65499 or 58875 protein or nucleic acid is detected in the biological sample. The term "biological sample" includes tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. A preferred biological sample is serum. The level of expression of the 65499 or 58875 gene can be measured in a number of ways, including, but not limited to: measuring the mRNA encoded by the 65499 or 58875 genes; measuring the amount of protein encoded by the

65499 or 58875 genes; or measuring the activity of the protein encoded by the 65499 or 58875 genes.

The level of mRNA corresponding to the 65499 or 58875 gene in a cell can be determined both by *in situ* and by *in vitro* formats.

5 The isolated mRNA can be used in hybridization or amplification assays that include, but are not limited to, Southern or Northern analyses, polymerase chain reaction analyses and probe arrays. One preferred diagnostic method for the detection of mRNA levels involves contacting the isolated mRNA with a nucleic acid molecule (probe) that can hybridize to the mRNA encoded by the gene being detected. The nucleic acid probe can be, 10 for example, a full-length 65499 or 58875 nucleic acid, such as the nucleic acid of SEQ ID NO:1, 3, 5, 6, or the DNA insert of any of the plasmids deposited with ATCC as Accession Numbers _____, or a portion thereof, such as an oligonucleotide of at least 7, 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to 65499 or 58875 mRNA or genomic DNA. Other suitable probes for 15 use in the diagnostic assays are described herein.

In one format, mRNA (or cDNA) is immobilized on a surface and contacted with the probes, for example by running the isolated mRNA on an agarose gel and transferring the mRNA from the gel to a membrane, such as nitrocellulose. In an alternative format, the probes are immobilized on a surface and the mRNA (or cDNA) is contacted with the 20 probes, for example, in a two-dimensional gene chip array. A skilled artisan can adapt known mRNA detection methods for use in detecting the level of mRNA encoded by the 65499 or 58875 genes.

The level of mRNA in a sample that is encoded by one of 65499 or 58875 can be evaluated with nucleic acid amplification, *e.g.*, by rtPCR (Mullis, 1987, U.S. Patent No. 25 4,683,202), ligase chain reaction (Barany, 1991, Proc. Natl. Acad. Sci. USA 88:189-193), self sustained sequence replication (Guatelli *et al.*, 1990, Proc. Natl. Acad. Sci. USA 87:1874-1878), transcriptional amplification system (Kwoh *et al.*, 1989, Proc. Natl. Acad. Sci. USA 86:1173-1177), Q-Beta Replicase (Lizardi *et al.*, 1988, Bio/Technology 6:1197), rolling circle replication (Lizardi *et al.*, U.S. Patent No. 5,854,033) or any other nucleic 30 acid amplification method, followed by the detection of the amplified molecules using techniques known in the art. As used herein, amplification primers are defined as being a pair of nucleic acid molecules that can anneal to 5' or 3' regions of a gene (plus and minus strands, respectively, or vice-versa) and contain a short region in between. In general,

amplification primers are from about 10 to 30 nucleotides in length and flank a region from about 50 to 200 nucleotides in length. Under appropriate conditions and with appropriate reagents, such primers permit the amplification of a nucleic acid molecule comprising the nucleotide sequence flanked by the primers.

5 For *in situ* methods, a cell or tissue sample can be prepared/processed and immobilized on a support, typically a glass slide, and then contacted with a probe that can hybridize to mRNA that encodes the 65499 or 58875 gene being analyzed.

 In another embodiment, the methods further contacting a control sample with a compound or agent capable of detecting 65499 or 58875 mRNA, or genomic DNA, and
10 comparing the presence of 65499 or 58875 mRNA or genomic DNA in the control sample with the presence of 65499 or 58875 mRNA or genomic DNA in the test sample.

 A variety of methods can be used to determine the level of protein encoded by 65499 or 58875. In general, these methods include contacting an agent that selectively binds to the protein, such as an antibody with a sample, to evaluate the level of protein in
15 the sample. In a preferred embodiment, the antibody bears a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (*e.g.*, Fab or F(ab')₂) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (*i.e.*, physically linking) a detectable substance to the probe or antibody, as well as indirect
20 labeling of the probe or antibody by reactivity with a detectable substance. Examples of detectable substances are provided herein.

 The detection methods can be used to detect 65499 or 58875 protein in a biological sample *in vitro* as well as *in vivo*. *In vitro* techniques for detection of 65499 or 58875 protein include enzyme linked immunosorbent assays (ELISAs), immunoprecipitations,
25 immunofluorescence, enzyme immunoassay (EIA), radioimmunoassay (RIA), and Western blot analysis. *In vivo* techniques for detection of 65499 or 58875 protein include introducing into a subject a labeled anti-65499 or 58875 antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

30 In another embodiment, the methods further include contacting the control sample with a compound or agent capable of detecting 65499 or 58875 protein, and comparing the

presence of 65499 or 58875 protein in the control sample with the presence of 65499 or 58875 protein in the test sample.

The invention also includes kits for detecting the presence of 65499 or 58875 in a biological sample. For example, the kit can include a compound or agent capable of
5 detecting 65499 or 58875 protein or mRNA in a biological sample; and a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect 65499 or 58875 protein or nucleic acid.

For antibody-based kits, the kit can include: (1) a first antibody (*e.g.*, attached to a solid support) which binds to a polypeptide corresponding to a marker of the invention;
10 and, optionally, (2) a second, different antibody which binds to either the polypeptide or the first antibody and is conjugated to a detectable agent.

For oligonucleotide-based kits, the kit can include: (1) an oligonucleotide, *e.g.*, a detectably labeled oligonucleotide, which hybridizes to a nucleic acid sequence encoding a polypeptide corresponding to a marker of the invention or (2) a pair of primers useful for
15 amplifying a nucleic acid molecule corresponding to a marker of the invention. The kit can also include a buffering agent, a preservative, or a protein stabilizing agent. The kit can also include components necessary for detecting the detectable agent (*e.g.*, an enzyme or a substrate). The kit can also contain a control sample or a series of control samples which can be assayed and compared to the test sample contained. Each component of the kit can
20 be enclosed within an individual container and all of the various containers can be within a single package, along with instructions for interpreting the results of the assays performed using the kit.

The diagnostic methods described herein can identify subjects having, or at risk of developing, a disease or disorder associated with misexpressed or aberrant or unwanted
25 65499 or 58875 expression or activity. As used herein, the term "unwanted" includes an unwanted phenomenon involved in a biological response such as pain or deregulated cell proliferation.

In one embodiment, a disease or disorder associated with aberrant or unwanted 65499 or 58875 expression or activity is identified. A test sample is obtained from a
30 subject and 65499 or 58875 protein or nucleic acid (*e.g.*, mRNA or genomic DNA) is evaluated, wherein the level, *e.g.*, the presence or absence, of 65499 or 58875 protein or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant or unwanted 65499 or 58875 expression or activity. As used

herein, a "test sample" refers to a biological sample obtained from a subject of interest, including a biological fluid (*e.g.*, serum), cell sample, or tissue.

The prognostic assays described herein can be used to determine whether a subject can be administered an agent (*e.g.*, an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with aberrant or unwanted 65499 or 58875 expression or activity. For example, such methods can be used to determine whether a subject can be effectively treated with an agent that modulates 65499 or 58875 expression or activity.

The methods of the invention can also be used to detect genetic alterations in a 65499 or 58875 gene, thereby determining if a subject with the altered gene is at risk for a disorder characterized by misregulation in 65499 or 58875 protein activity or nucleic acid expression, such as a disorder associated with bone metabolism, an immune disorder, a neurodegenerative disorder, a disorders involving the trachea, or a cardiovascular disorder. In preferred embodiments, the methods include detecting, in a sample from the subject, the presence or absence of a genetic alteration characterized by at least one of an alteration affecting the integrity of a gene encoding a 65499 or 58875-protein, or the mis-expression of the 65499 or 58875 gene. For example, such genetic alterations can be detected by ascertaining the existence of at least one of 1) a deletion of one or more nucleotides from a 65499 or 58875 gene; 2) an addition of one or more nucleotides to a 65499 or 58875 gene; 3) a substitution of one or more nucleotides of a 65499 or 58875 gene, 4) a chromosomal rearrangement of a 65499 or 58875 gene; 5) an alteration in the level of a messenger RNA transcript of a 65499 or 58875 gene, 6) aberrant modification of a 65499 or 58875 gene, such as of the methylation pattern of the genomic DNA, 7) the presence of a non-wild type splicing pattern of a messenger RNA transcript of a 65499 or 58875 gene, 8) a non-wild type level of a 65499 or 58875-protein, 9) allelic loss of a 65499 or 58875 gene, and 10) inappropriate post-translational modification of a 65499 or 58875-protein.

An alteration can be detected without a probe/primer in a polymerase chain reaction, such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR), the latter of which can be particularly useful for detecting point mutations in the 65499 or 58875-gene. This method can include the steps of collecting a sample of cells from a subject, isolating nucleic acid (*e.g.*, genomic, mRNA or both) from the sample, contacting the nucleic acid sample with one or more primers which specifically hybridize to a 65499 or 58875 gene under conditions such that hybridization and amplification of the

65499 or 58875-gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for
5 detecting mutations described herein.

Alternative amplification methods include: self sustained sequence replication (Guatelli, J.C. *et al.*, (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional amplification system (Kwoh, D. Y. *et al.*, (1989) *Proc. Natl. Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase (Lizardi, P.M. *et al.* (1988) *Bio-Technology* 6:1197), or other nucleic acid
10 amplification methods, followed by the detection of the amplified molecules using techniques known to those of skill in the art.

In another embodiment, mutations in a 65499 or 58875 gene from a sample cell can be identified by detecting alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more
15 restriction endonucleases, and fragment length sizes are determined, *e.g.*, by gel electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (see, for example, U.S. Patent No. 5,498,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

20 In other embodiments, genetic mutations in 65499 or 58875 can be identified by hybridizing a sample and control nucleic acids, *e.g.*, DNA or RNA, two dimensional arrays, *e.g.*, chip based arrays. Such arrays include a plurality of addresses, each of which is positionally distinguishable from the other. A different probe is located at each address of the plurality. The arrays can have a high density of addresses, *e.g.*, can contain hundreds or
25 thousands of oligonucleotides probes (Cronin, M. T. *et al.* (1996) *Human Mutation* 7: 244-255; Kozal, M. J. *et al.* (1996) *Nature Medicine* 2: 753-759). For example, genetic mutations in 65499 or 58875 can be identified in two dimensional arrays containing light-generated DNA probes as described in Cronin, M. T. *et al. supra*. Briefly, a first hybridization array of probes can be used to scan through long stretches of DNA in a
30 sample and control to identify base changes between the sequences by making linear arrays of sequential overlapping probes. This step allows the identification of point mutations. This step is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants

or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the 65499 or 58875 gene and detect mutations by
5 comparing the sequence of the sample 65499 or 58875 with the corresponding wild-type (control) sequence. Automated sequencing procedures can be utilized when performing the diagnostic assays ((1995) *Biotechniques* 19:448), including sequencing by mass spectrometry.

Other methods for detecting mutations in the 65499 or 58875 gene include methods
10 in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes (Myers *et al.* (1985) *Science* 230:1242; Cotton *et al.* (1988) *Proc. Natl Acad Sci USA* 85:4397; Saleeba *et al.* (1992) *Methods Enzymol.* 217:286-295).

In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA
15 mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in 65499 or 58875 cDNAs obtained from samples of cells. For example, the mutY enzyme of *E. coli* cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches (Hsu *et al.* (1994) *Carcinogenesis* 15:1657-1662; U.S. Patent No. 5,459,039).

In other embodiments, alterations in electrophoretic mobility will be used to
20 identify mutations in 65499 or 58875 genes. For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids (Orita *et al.* (1989) *Proc Natl. Acad. Sci USA*: 86:2766, see also Cotton (1993) *Mutat. Res.* 285:125-144; and Hayashi (1992) *Genet. Anal.*
25 *Tech. Appl.* 9:73-79). Single-stranded DNA fragments of sample and control 65499 or 58875 nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may
30 be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In a preferred embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility (Keen *et al.* (1991) *Trends Genet* 7:5).

In yet another embodiment, the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE) (Myers *et al.* (1985) *Nature* 313:495). When DGGE is used as the method of analysis, DNA will be modified to insure that it does not
5 completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA (Rosenbaum and Reissner (1987) *Biophys Chem* 265:12753).

Examples of other techniques for detecting point mutations include, but are not
10 limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension (Saiki *et al.* (1986) *Nature* 324:163); Saiki *et al.* (1989) *Proc. Natl. Acad. Sci USA* 86:6230).

Alternatively, allele specific amplification technology which depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides
15 used as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization) (Gibbs *et al.* (1989) *Nucleic Acids Res.* 17:2437-2448) or at the extreme 3' end of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (Prossner (1993) *Tibtech* 11:238). In addition it may be desirable to introduce a novel
20 restriction site in the region of the mutation to create cleavage-based detection (Gasparini *et al.* (1992) *Mol. Cell Probes* 6:1). It is anticipated that in certain embodiments amplification may also be performed using Taq ligase for amplification (Barany (1991) *Proc. Natl. Acad. Sci USA* 88:189). In such cases, ligation will occur only if there is a perfect match at the 3' end of the 5' sequence making it possible to detect the presence of a known mutation at a
25 specific site by looking for the presence or absence of amplification.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, *e.g.*, in clinical settings to diagnose patients exhibiting symptoms or family history of a disease or illness involving a 65499 or
30 58875 gene.

Use of 65499 or 58875 Molecules as Surrogate Markers

The 65499 or 58875 molecules of the invention are also useful as markers of disorders or disease states, as markers for precursors of disease states, as markers for predisposition of disease states, as markers of drug activity, or as markers of the pharmacogenomic profile of a subject. Using the methods described herein, the presence, 5 absence and/or quantity of the 65499 or 58875 molecules of the invention may be detected, and may be correlated with one or more biological states *in vivo*. For example, the 65499 or 58875 molecules of the invention may serve as surrogate markers for one or more disorders or disease states or for conditions leading up to disease states. As used herein, a “surrogate marker” is an objective biochemical marker which correlates with the absence or 10 presence of a disease or disorder, or with the progression of a disease or disorder (*e.g.*, with the presence or absence of a tumor). The presence or quantity of such markers is independent of the disease. Therefore, these markers may serve to indicate whether a particular course of treatment is effective in lessening a disease state or disorder. Surrogate markers are of particular use when the presence or extent of a disease state or disorder is 15 difficult to assess through standard methodologies (*e.g.*, early stage tumors), or when an assessment of disease progression is desired before a potentially dangerous clinical endpoint is reached (*e.g.*, an assessment of cardiovascular disease may be made using cholesterol levels as a surrogate marker, and an analysis of HIV infection may be made using HIV RNA levels as a surrogate marker, well in advance of the undesirable clinical 20 outcomes of myocardial infarction or fully-developed AIDS). Examples of the use of surrogate markers in the art include: Koomen *et al.* (2000) *J. Mass. Spectrom.* 35: 258-264; and James (1994) *AIDS Treatment News Archive* 209.

The 65499 or 58875 molecules of the invention are also useful as pharmacodynamic markers. As used herein, a “pharmacodynamic marker” is an objective biochemical marker 25 which correlates specifically with drug effects. The presence or quantity of a pharmacodynamic marker is not related to the disease state or disorder for which the drug is being administered; therefore, the presence or quantity of the marker is indicative of the presence or activity of the drug in a subject. For example, a pharmacodynamic marker may be indicative of the concentration of the drug in a biological tissue, in that the marker is 30 either expressed or transcribed or not expressed or transcribed in that tissue in relationship to the level of the drug. In this fashion, the distribution or uptake of the drug may be monitored by the pharmacodynamic marker. Similarly, the presence or quantity of the pharmacodynamic marker may be related to the presence or quantity of the metabolic

product of a drug, such that the presence or quantity of the marker is indicative of the relative breakdown rate of the drug *in vivo*. Pharmacodynamic markers are of particular use in increasing the sensitivity of detection of drug effects, particularly when the drug is administered in low doses. Since even a small amount of a drug may be sufficient to

5 activate multiple rounds of marker (e.g., a 65499 or 58875 marker) transcription or expression, the amplified marker may be in a quantity which is more readily detectable than the drug itself. Also, the marker may be more easily detected due to the nature of the marker itself; for example, using the methods described herein, anti-65499 or 58875 antibodies may be employed in an immune-based detection system for a 65499 or 58875

10 protein marker, or 65499- or 58875-specific radiolabeled probes may be used to detect a 65499 or 58875 mRNA marker. Furthermore, the use of a pharmacodynamic marker may offer mechanism-based prediction of risk due to drug treatment beyond the range of possible direct observations. Examples of the use of pharmacodynamic markers in the art include: Matsuda *et al.* US 6,033,862; Hattis *et al.* (1991) *Env. Health Perspect.* 90: 229-

15 238; Schentag (1999) *Am. J. Health-Syst. Pharm.* 56 Suppl. 3: S21-S24; and Nicolau (1999) *Am. J. Health-Syst. Pharm.* 56 Suppl. 3: S16-S20.

The 65499 or 58875 molecules of the invention are also useful as pharmacogenomic markers. As used herein, a "pharmacogenomic marker" is an objective biochemical marker which correlates with a specific clinical drug response or susceptibility in a subject (see,

20 e.g., McLeod *et al.* (1999) *Eur. J. Cancer* 35(12): 1650-1652). The presence or quantity of the pharmacogenomic marker is related to the predicted response of the subject to a specific drug or class of drugs prior to administration of the drug. By assessing the presence or quantity of one or more pharmacogenomic markers in a subject, a drug therapy which is most appropriate for the subject, or which is predicted to have a greater degree of success,

25 may be selected. For example, based on the presence or quantity of RNA, or protein (e.g., 65499 or 58875 protein or RNA) for specific tumor markers in a subject, a drug or course of treatment may be selected that is optimized for the treatment of the specific tumor likely to be present in the subject. Similarly, the presence or absence of a specific sequence mutation in 65499 or 58875 DNA may correlate 65499 or 58875 drug response. The use of

30 pharmacogenomic markers therefore permits the application of the most appropriate treatment for each subject without having to administer the therapy.

Pharmaceutical Compositions

The nucleic acid and polypeptides, fragments thereof, as well as anti-65499 or 58875 antibodies (also referred to herein as "active compounds") of the invention can be incorporated into pharmaceutical compositions. Such compositions typically include the nucleic acid molecule, protein, or antibody and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" includes solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration.

10 Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, 15 intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or 20 dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous 25 solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It should be 30 stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable

mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

10 Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules, *e.g.*, gelatin capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD₅₀/ED₅₀. Compounds which exhibit high therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such

compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC₅₀ (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

As defined herein, a therapeutically effective amount of protein or polypeptide (*i.e.*, an effective dosage) ranges from about 0.001 to 30 mg/kg body weight, preferably about 0.01 to 25 mg/kg body weight, more preferably about 0.1 to 20 mg/kg body weight, and even more preferably about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4 to 7 mg/kg, or 5 to 6 mg/kg body weight. The protein or polypeptide can be administered one time per week for between about 1 to 10 weeks, preferably between 2 to 8 weeks, more preferably between about 3 to 7 weeks, and even more preferably for about 4, 5, or 6 weeks. The skilled artisan will appreciate that certain factors may influence the dosage and timing required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a protein, polypeptide, or antibody can include a single treatment or, preferably, can include a series of treatments.

For antibodies, the preferred dosage is 0.1 mg/kg of body weight (generally 10 mg/kg to 20 mg/kg). If the antibody is to act in the brain, a dosage of 50 mg/kg to 100 mg/kg is usually appropriate. Generally, partially human antibodies and fully human antibodies have a longer half-life within the human body than other antibodies. Accordingly, lower dosages and less frequent administration is often possible. Modifications such as lipidation can be used to stabilize antibodies and to enhance uptake and tissue penetration (*e.g.*, into the brain). A method for lipidation of antibodies is

described by Cruikshank *et al.* ((1997) *J. Acquired Immune Deficiency Syndromes and Human Retrovirology* 14:193).

The present invention encompasses agents which modulate expression or activity. An agent may, for example, be a small molecule. For example, such small molecules include, but are not limited to, peptides, peptidomimetics (*e.g.*, peptoids), amino acids, amino acid analogs, polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs, organic or inorganic compounds (*i.e.*, including heteroorganic and organometallic compounds) having a molecular weight less than about 10,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds.

Exemplary doses include milligram or microgram amounts of the small molecule per kilogram of subject or sample weight (*e.g.*, about 1 microgram per kilogram to about 500 milligrams per kilogram, about 100 micrograms per kilogram to about 5 milligrams per kilogram, or about 1 microgram per kilogram to about 50 micrograms per kilogram. It is furthermore understood that appropriate doses of a small molecule depend upon the potency of the small molecule with respect to the expression or activity to be modulated. When one or more of these small molecules is to be administered to an animal (*e.g.*, a human) in order to modulate expression or activity of a polypeptide or nucleic acid of the invention, a physician, veterinarian, or researcher may, for example, prescribe a relatively low dose at first, subsequently increasing the dose until an appropriate response is obtained. In addition, it is understood that the specific dose level for any particular animal subject will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, gender, and diet of the subject, the time of administration, the route of administration, the rate of excretion, any drug combination, and the degree of expression or activity to be modulated.

An antibody (or fragment thereof) may be conjugated to a therapeutic moiety such as a cytotoxin, a therapeutic agent or a radioactive metal ion. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione,

mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine),
5 alkylating agents (*e.g.*, mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.*, daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.*, dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin
10 (AMC)), and anti-mitotic agents (*e.g.*, vincristine and vinblastine).

The conjugates of the invention can be used for modifying a given biological response, the drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A,
15 pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, .alpha.-interferon, .beta.-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophase colony stimulating factor ("GM-CSF"), granulocyte colony
20 stimulating factor ("G-CSF"), or other growth factors.

Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980.

The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example,
25 intravenous injection, local administration (see U.S. Patent 5,328,470) or by stereotactic injection (see *e.g.*, Chen *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be
30 produced intact from recombinant cells, *e.g.*, retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

Methods of Treatment

The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant or unwanted 65499 or 58875 expression or activity. With regards to both prophylactic and therapeutic methods of treatment, such treatments may be specifically tailored or modified, based on knowledge obtained from the field of pharmacogenomics. As used herein, the term "treatment" is defined as the application or administration of a therapeutic agent to a patient, or application or administration of a therapeutic agent to an isolated tissue or cell line from a patient, who has a disease, a symptom of disease or a predisposition toward a disease, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve or affect the disease, the symptoms of disease or the predisposition toward disease. A therapeutic agent includes, but is not limited to, small molecules, peptides, antibodies, ribozymes and antisense oligonucleotides.

"Pharmacogenomics", as used herein, refers to the application of genomics technologies such as gene sequencing, statistical genetics, and gene expression analysis to drugs in clinical development and on the market. More specifically, the term refers the study of how a patient's genes determine his or her response to a drug (e.g., a patient's "drug response phenotype", or "drug response genotype".) Thus, another aspect of the invention provides methods for tailoring an individual's prophylactic or therapeutic treatment with either the 65499 or 58875 molecules of the present invention or 65499 or 58875 modulators according to that individual's drug response genotype. Pharmacogenomics allows a clinician or physician to target prophylactic or therapeutic treatments to patients who will most benefit from the treatment and to avoid treatment of patients who will experience toxic drug-related side effects.

In one aspect, the invention provides a method for preventing in a subject, a disease or condition associated with an aberrant or unwanted 65499 or 58875 expression or activity, by administering to the subject a 65499 or 58875 or an agent which modulates 65499 or 58875 expression or at least one 65499 or 58875 activity. Subjects at risk for a disease which is caused or contributed to by aberrant or unwanted 65499 or 58875 expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the 65499 or 58875 aberrance, such

that a disease or disorder is prevented or, alternatively, delayed in its progression.

Depending on the type of 65499 or 58875 aberrance, for example, a 65499 or 58875, 65499 or 58875 agonist or 65499 or 58875 antagonist agent can be used for treating the subject.

The appropriate agent can be determined based on screening assays described herein.

- 5 It is possible that some 65499 or 58875 disorders can be caused, at least in part, by an abnormal level of gene product, or by the presence of a gene product exhibiting abnormal activity. As such, the reduction in the level and/or activity of such gene products would bring about the amelioration of disorder symptoms.

- The 65499 or 58875 molecules can act as novel diagnostic targets and therapeutic
10 agents for controlling one or more of cellular proliferative and/or differentiative disorders, disorders associated with bone metabolism, hematopoietic disorders, cardiovascular disorders, including endothelial cell disorders, blood vessel disorders, brain disorders, and hormonal disorders, as described above, as well as disorders associated with immune disorders, liver disorders, viral diseases, platelet disorders, and pain or metabolic disorders.

- 15 The 65499 or 58875 nucleic acid and protein of the invention can be used to treat and/or diagnose a variety of immune, *e.g.*, inflammatory (*e.g.* respiratory inflammatory) disorders. Examples immune and inflammatory disorders or diseases include, but are not limited to, autoimmune diseases (including, for example, diabetes mellitus, arthritis (including rheumatoid arthritis, juvenile rheumatoid arthritis, osteoarthritis, psoriatic
20 arthritis), multiple sclerosis, encephalomyelitis, myasthenia gravis, systemic lupus erythematosus, autoimmune thyroiditis, dermatitis (including atopic dermatitis and eczematous dermatitis), psoriasis, Sjögren's Syndrome, inflammatory bowel disease, *e.g.* Crohn's disease and ulcerative colitis, aphthous ulcer, iritis, conjunctivitis, keratoconjunctivitis, asthma, allergic asthma, chronic obstructive pulmonary disease,
25 cutaneous lupus erythematosus, scleroderma, vaginitis, proctitis, drug eruptions, leprosy reversal reactions, erythema nodosum leprosum, autoimmune uveitis, allergic encephalomyelitis, acute necrotizing hemorrhagic encephalopathy, idiopathic bilateral progressive sensorineural hearing loss, aplastic anemia, pure red cell anemia, idiopathic thrombocytopenia, polychondritis, Wegener's granulomatosis, chronic active hepatitis,
30 Stevens-Johnson syndrome, idiopathic sprue, lichen planus, Graves' disease, sarcoidosis, primary biliary cirrhosis, uveitis posterior, and interstitial lung fibrosis), graft-versus-host disease, cases of transplantation, and allergy such as, atopic allergy.

Disorders which can be treated or diagnosed by methods described herein include, but are not limited to, disorders associated with an accumulation in the liver of fibrous tissue, such as that resulting from an imbalance between production and degradation of the extracellular matrix accompanied by the collapse and condensation of preexisting fibers.

5 The methods described herein can be used to diagnose or treat hepatocellular necrosis or injury induced by a wide variety of agents including processes which disturb homeostasis, such as an inflammatory process, tissue damage resulting from toxic injury or altered hepatic blood flow, and infections (*e.g.*, bacterial, viral and parasitic). For example, the methods can be used for the early detection of hepatic injury, such as portal hypertension or
10 hepatic fibrosis. In addition, the methods can be employed to detect liver fibrosis attributed to inborn errors of metabolism, for example, fibrosis resulting from a storage disorder such as Gaucher's disease (lipid abnormalities) or a glycogen storage disease, A1-antitrypsin deficiency; a disorder mediating the accumulation (*e.g.*, storage) of an exogenous substance, for example, hemochromatosis (iron-overload syndrome) and copper storage
15 diseases (Wilson's disease), disorders resulting in the accumulation of a toxic metabolite (*e.g.*, tyrosinemia, fructosemia and galactosemia) and peroxisomal disorders (*e.g.*, Zellweger syndrome). Additionally, the methods described herein can be useful for the early detection and treatment of liver injury associated with the administration of various chemicals or drugs, such as for example, methotrexate, isoniazid, oxyphenisatin,
20 methyl dopa, chlorpromazine, tolbutamide or alcohol, or which represents a hepatic manifestation of a vascular disorder such as obstruction of either the intrahepatic or extrahepatic bile flow or an alteration in hepatic circulation resulting, for example, from chronic heart failure, veno-occlusive disease, portal vein thrombosis or Budd-Chiari syndrome.

25 Additionally, 65499 or 58875 molecules can play an important role in the etiology of certain viral diseases, including but not limited to Hepatitis B, Hepatitis C and Herpes Simplex Virus (HSV). Modulators of 65499 or 58875 activity could be used to control viral diseases. The modulators can be used in the treatment and/or diagnosis of viral infected tissue or virus-associated tissue fibrosis, especially liver and liver fibrosis. Also,
30 65499 or 58875 modulators can be used in the treatment and/or diagnosis of virus-associated carcinoma, especially hepatocellular cancer.

Additionally, 65499 or 58875 can play an important role in the regulation of metabolism or pain disorders. Diseases of metabolic imbalance include, but are not limited

to, obesity, anorexia nervosa, cachexia, lipid disorders, and diabetes. Examples of pain disorders include, but are not limited to, pain response elicited during various forms of tissue injury, *e.g.*, inflammation, infection, and ischemia, usually referred to as hyperalgesia (described in, for example, Fields, H.L. (1987) *Pain*, New York:McGraw-Hill); pain
5 associated with musculoskeletal disorders, *e.g.*, joint pain; tooth pain; headaches; pain associated with surgery; pain related to irritable bowel syndrome; or chest pain.

As discussed, successful treatment of 65499 or 58875 disorders can be brought about by techniques that serve to inhibit the expression or activity of target gene products. For example, compounds, *e.g.*, an agent identified using an assays described above, that
10 proves to exhibit negative modulatory activity, can be used in accordance with the invention to prevent and/or ameliorate symptoms of 65499 or 58875 disorders. Such molecules can include, but are not limited to peptides, phosphopeptides, small organic or inorganic molecules, or antibodies (including, for example, polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain antibodies, and FAb, F(ab')₂ and FAb
15 expression library fragments, scFV molecules, and epitope-binding fragments thereof).

Further, antisense and ribozyme molecules that inhibit expression of the target gene can also be used in accordance with the invention to reduce the level of target gene expression, thus effectively reducing the level of target gene activity. Still further, triple helix molecules can be utilized in reducing the level of target gene activity. Antisense,
20 ribozyme and triple helix molecules are discussed above.

It is possible that the use of antisense, ribozyme, and/or triple helix molecules to reduce or inhibit mutant gene expression can also reduce or inhibit the transcription (triple helix) and/or translation (antisense, ribozyme) of mRNA produced by normal target gene alleles, such that the concentration of normal target gene product present can be lower than
25 is necessary for a normal phenotype. In such cases, nucleic acid molecules that encode and express target gene polypeptides exhibiting normal target gene activity can be introduced into cells via gene therapy method. Alternatively, in instances in that the target gene encodes an extracellular protein, it can be preferable to co-administer normal target gene protein into the cell or tissue in order to maintain the requisite level of cellular or tissue
30 target gene activity.

Another method by which nucleic acid molecules may be utilized in treating or preventing a disease characterized by 65499 or 58875 expression is through the use of aptamer molecules specific for 65499 or 58875 protein. Aptamers are nucleic acid

molecules having a tertiary structure which permits them to specifically bind to protein ligands (see, e.g., Osborne, *et al. Curr. Opin. Chem Biol.* 1997, 1(1): 5-9; and Patel, D. J. *Curr Opin Chem Biol* 1997 Jun;1(1):32-46). Since nucleic acid molecules may in many cases be more conveniently introduced into target cells than therapeutic protein molecules
5 may be, aptamers offer a method by which 65499 or 58875 protein activity may be specifically decreased without the introduction of drugs or other molecules which may have pluripotent effects.

Antibodies can be generated that are both specific for target gene product and that reduce target gene product activity. Such antibodies may, therefore, be administered in
10 instances whereby negative modulatory techniques are appropriate for the treatment of 65499 or 58875 disorders. For a description of antibodies, see the Antibody section above.

In circumstances wherein injection of an animal or a human subject with a 65499 or 58875 protein or epitope for stimulating antibody production is harmful to the subject, it is possible to generate an immune response against 65499 or 58875 through the use of anti-
15 idiotypic antibodies (see, for example, Herlyn, D. *Ann Med* 1999;31(1):66-78; and Bhattacharya-Chatterjee, M., and Foon, K. A. *Cancer Treat Res* 1998;94:51-68). If an anti-idiotypic antibody is introduced into a mammal or human subject, it should stimulate the production of anti-anti-idiotypic antibodies, which should be specific to the 65499 or 58875 protein. Vaccines directed to a disease characterized by 65499 or 58875 expression may
20 also be generated in this fashion.

In instances where the target antigen is intracellular and whole antibodies are used, internalizing antibodies may be preferred. Lipofectin or liposomes can be used to deliver the antibody or a fragment of the Fab region that binds to the target antigen into cells. Where fragments of the antibody are used, the smallest inhibitory fragment that binds to the
25 target antigen is preferred. For example, peptides having an amino acid sequence corresponding to the Fv region of the antibody can be used. Alternatively, single chain neutralizing antibodies that bind to intracellular target antigens can also be administered. Such single chain antibodies can be administered, for example, by expressing nucleotide sequences encoding single-chain antibodies within the target cell population (see e.g.,
30 Marasco *et al.* (1993, *Proc. Natl. Acad. Sci. USA* 90:7889-7893).

The identified compounds that inhibit target gene expression, synthesis and/or activity can be administered to a patient at therapeutically effective doses to prevent, treat

or ameliorate 65499 or 58875 disorders. A therapeutically effective dose refers to that amount of the compound sufficient to result in amelioration of symptoms of the disorders.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining
5 the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD₅₀/ED₅₀. Compounds that exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects can be used, care should be taken to design a delivery system that targets such
10 compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED₅₀ with little or
15 no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose can be formulated in animal models to achieve a circulating plasma concentration range that includes the IC₅₀ (*i.e.*, the concentration of the test compound that
20 achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma can be measured, for example, by high performance liquid chromatography.

Another example of determination of effective dose for an individual is the ability to directly assay levels of "free" and "bound" compound in the serum of the test subject.
25 Such assays may utilize antibody mimics and/or "biosensors" that have been created through molecular imprinting techniques. The compound which is able to modulate 65499 or 58875 activity is used as a template, or "imprinting molecule", to spatially organize polymerizable monomers prior to their polymerization with catalytic reagents. The subsequent removal of the imprinted molecule leaves a polymer matrix which contains a
30 repeated "negative image" of the compound and is able to selectively rebind the molecule under biological assay conditions. A detailed review of this technique can be seen in Ansell, R. J. *et al* (1996) *Current Opinion in Biotechnology* 7:89-94 and in Shea, K. J. (1994) *Trends in Polymer Science* 2:166-173. Such "imprinted" affinity matrixes are amenable to

ligand-binding assays, whereby the immobilized monoclonal antibody component is replaced by an appropriately imprinted matrix. An example of the use of such matrixes in this way can be seen in Vlatakis, G. *et al* (1993) *Nature* 361:645-647. Through the use of isotope-labeling, the “free” concentration of compound which modulates the expression or activity of 65499 or 58875 can be readily monitored and used in calculations of IC₅₀.

Such “imprinted” affinity matrixes can also be designed to include fluorescent groups whose photon-emitting properties measurably change upon local and selective binding of target compound. These changes can be readily assayed in real time using appropriate fiberoptic devices, in turn allowing the dose in a test subject to be quickly optimized based on its individual IC₅₀. An rudimentary example of such a “biosensor” is discussed in Kriz, D. *et al* (1995) *Analytical Chemistry* 67:2142-2144.

Another aspect of the invention pertains to methods of modulating 65499 or 58875 expression or activity for therapeutic purposes. Accordingly, in an exemplary embodiment, the modulatory method of the invention involves contacting a cell with a 65499 or 58875 or agent that modulates one or more of the activities of 65499 or 58875 protein activity associated with the cell. An agent that modulates 65499 or 58875 protein activity can be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring target molecule of a 65499 or 58875 protein (*e.g.*, a 65499 or 58875 substrate or receptor), a 65499 or 58875 antibody, a 65499 or 58875 agonist or antagonist, a peptidomimetic of a 65499 or 58875 agonist or antagonist, or other small molecule.

In one embodiment, the agent stimulates one or more 65499 or 58875 activities. Examples of such stimulatory agents include active 65499 or 58875 protein and a nucleic acid molecule encoding 65499 or 58875. In another embodiment, the agent inhibits one or more 65499 or 58875 activities. Examples of such inhibitory agents include antisense 65499 or 58875 nucleic acid molecules, anti-65499 or 58875 antibodies, and 65499 or 58875 inhibitors. These modulatory methods can be performed *in vitro* (*e.g.*, by culturing the cell with the agent) or, alternatively, *in vivo* (*e.g.*, by administering the agent to a subject). As such, the present invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant or unwanted expression or activity of a 65499 or 58875 protein or nucleic acid molecule. In one embodiment, the method involves administering an agent (*e.g.*, an agent identified by a screening assay described herein), or combination of agents that modulates (*e.g.*, upregulates or downregulates) 65499 or 58875 expression or activity. In another embodiment, the method involves administering a 65499

or 58875 protein or nucleic acid molecule as therapy to compensate for reduced, aberrant, or unwanted 65499 or 58875 expression or activity.

Stimulation of 65499 or 58875 activity is desirable in situations in which 65499 or 58875 is abnormally downregulated and/or in which increased 65499 or 58875 activity is likely to have a beneficial effect. For example, stimulation of 65499 or 58875 activity is desirable in situations in which a 65499 or 58875 is downregulated and/or in which increased 65499 or 58875 activity is likely to have a beneficial effect. Likewise, inhibition of 65499 or 58875 activity is desirable in situations in which 65499 or 58875 is abnormally upregulated and/or in which decreased 65499 or 58875 activity is likely to have a beneficial effect.

Pharmacogenomics

The 65499 or 58875 molecules of the present invention, as well as agents, or modulators which have a stimulatory or inhibitory effect on 65499 or 58875 activity (*e.g.*, 65499 or 58875 gene expression) as identified by a screening assay described herein can be administered to individuals to treat (prophylactically or therapeutically) 65499 or 58875-associated disorders associated with aberrant or unwanted 65499 or 58875 activity (*e.g.*, disorders associated with cellular proliferative and/or differentiative disorders, disorders associated with bone metabolism, hematopoietic disorders, cardiovascular disorders, including endothelial cell disorders, brain disorders, a neurodegenerative disorder, and hormonal disorders). In conjunction with such treatment, pharmacogenomics may be considered. "Pharmacogenomics", as used herein, refers to the application of genomics technologies such as gene sequencing, statistical genetics, and gene expression analysis to drugs in clinical development and on the market. More specifically, the term refers the study of how a patient's genes determine his or her response to a drug (*e.g.*, a patient's "drug response phenotype", or "drug response genotype".) Thus, another aspect of the invention provides methods for tailoring an individual's prophylactic or therapeutic treatment with either the 65499 or 58875 molecules of the present invention or 65499 or 58875 modulators according to that individual's drug response genotype.

Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See, for example, Eichelbaum, M. *et al.* (1996) *Clin. Exp. Pharmacol. Physiol.* 23(10-11):983-985 and Linder, M. W. *et al.* (1997) *Clin. Chem.* 43(2):254-266. In general, two types

of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare genetic defects or as naturally-occurring polymorphisms.

Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, a physician or clinician may consider applying knowledge obtained in relevant pharmacogenomics studies in determining whether to administer a 65499 or 58875 molecule or 65499 or 58875 modulator as well as tailoring the dosage and/or therapeutic regimen of treatment with a 65499 or 58875 molecule or 65499 or 58875 modulator.

One pharmacogenomics approach to identifying genes that predict drug response, known as "a genome-wide association", relies primarily on a high-resolution map of the human genome consisting of already known gene-related markers (*e.g.*, a "bi-allelic" gene marker map which consists of 60,000-100,000 polymorphic or variable sites on the human genome, each of which has two variants.) Such a high-resolution genetic map can be compared to a map of the genome of each of a statistically significant number of patients taking part in a Phase II/III drug trial to identify markers associated with a particular observed drug response or side effect. Alternatively, such a high resolution map can be generated from a combination of some ten-million known single nucleotide polymorphisms (SNPs) in the human genome. As used herein, a "SNP" is a common alteration that occurs in a single nucleotide base in a stretch of DNA. For example, a SNP may occur once per every 1000 bases of DNA. A SNP may be involved in a disease process, however, the vast majority may not be disease-associated. Given a genetic map based on the occurrence of such SNPs, individuals can be grouped into genetic categories depending on a particular pattern of SNPs in their individual genome. In such a manner, treatment regimens can be tailored to groups of genetically similar individuals, taking into account traits that may be common among such genetically similar individuals.

Alternatively, a method termed the "candidate gene approach", can be utilized to identify genes that predict drug response. According to this method, if a gene that encodes a drug's target is known (*e.g.*, a 65499 or 58875 protein of the present invention), all common variants of that gene can be fairly easily identified in the population and it can be

determined if having one version of the gene versus another is associated with a particular drug response.

Alternatively, a method termed the "gene expression profiling", can be utilized to identify genes that predict drug response. For example, the gene expression of an animal dosed with a drug (*e.g.*, a 65499 or 58875 molecule or 65499 or 58875 modulator of the present invention) can give an indication whether gene pathways related to toxicity have been turned on.

Information generated from more than one of the above pharmacogenomics approaches can be used to determine appropriate dosage and treatment regimens for prophylactic or therapeutic treatment of an individual. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with a 65499 or 58875 molecule or 65499 or 58875 modulator, such as a modulator identified by one of the exemplary screening assays described herein.

The present invention further provides methods for identifying new agents, or combinations, that are based on identifying agents that modulate the activity of one or more of the gene products encoded by one or more of the 65499 or 58875 genes of the present invention, wherein these products may be associated with resistance of the cells to a therapeutic agent. Specifically, the activity of the proteins encoded by the 65499 or 58875 genes of the present invention can be used as a basis for identifying agents for overcoming agent resistance. By blocking the activity of one or more of the resistance proteins, target cells, *e.g.*, bone cells, will become sensitive to treatment with an agent that the unmodified target cells were resistant to.

Monitoring the influence of agents (*e.g.*, drugs) on the expression or activity of a 65499 or 58875 protein can be applied in clinical trials. For example, the effectiveness of an agent determined by a screening assay as described herein to increase 65499 or 58875 gene expression, protein levels, or upregulate 65499 or 58875 activity, can be monitored in clinical trials of subjects exhibiting decreased 65499 or 58875 gene expression, protein levels, or downregulated 65499 or 58875 activity. Alternatively, the effectiveness of an agent determined by a screening assay to decrease 65499 or 58875 gene expression, protein levels, or downregulate 65499 or 58875 activity, can be monitored in clinical trials of subjects exhibiting increased 65499 or 58875 gene expression, protein levels, or upregulated 65499 or 58875 activity. In such clinical trials, the expression or activity of a

65499 or 58875 gene, and preferably, other genes that have been implicated in, for example, a 65499 or 58875-associated disorder can be used as a “read out” or markers of the phenotype of a particular cell.

5 Other Embodiments

In another aspect, the invention features, a method of analyzing a plurality of capture probes. The method can be used, *e.g.*, to analyze gene expression. The method includes: providing a two dimensional array having a plurality of addresses, each address of the plurality being positionally distinguishable from each other address of the plurality, and each address of the plurality having a unique capture probe, *e.g.*, a nucleic acid or
10 peptide sequence; contacting the array with a 65499 or 58875, preferably purified, nucleic acid, preferably purified, polypeptide, preferably purified, or antibody, and thereby evaluating the plurality of capture probes. Binding, *e.g.*, in the case of a nucleic acid, hybridization with a capture probe at an address of the plurality, is detected, *e.g.*, by signal
15 generated from a label attached to the 65499 or 58875 nucleic acid, polypeptide, or antibody.

The capture probes can be a set of nucleic acids from a selected sample, *e.g.*, a sample of nucleic acids derived from a control or non-stimulated tissue or cell.

The method can include contacting the 65499 or 58875 nucleic acid, polypeptide, or
20 antibody with a first array having a plurality of capture probes and a second array having a different plurality of capture probes. The results of each hybridization can be compared, *e.g.*, to analyze differences in expression between a first and second sample. The first plurality of capture probes can be from a control sample, *e.g.*, a wild type, normal, or non-diseased, non-stimulated, sample, *e.g.*, a biological fluid, tissue, or cell sample. The second
25 plurality of capture probes can be from an experimental sample, *e.g.*, a mutant type, at risk, disease-state or disorder-state, or stimulated, sample, *e.g.*, a biological fluid, tissue, or cell sample.

The plurality of capture probes can be a plurality of nucleic acid probes each of which specifically hybridizes, with an allele of 65499 or 58875. Such methods can be used
30 to diagnose a subject, *e.g.*, to evaluate risk for a disease or disorder, to evaluate suitability of a selected treatment for a subject, to evaluate whether a subject has a disease or disorder. 65499 or 58875 is associated with bone metabolism, thus it is useful for evaluating bone disorders.

The method can be used to detect SNPs, as described above.

In another aspect, the invention features, a method of analyzing a plurality of probes. The method is useful, *e.g.*, for analyzing gene expression. The method includes: providing a two dimensional array having a plurality of addresses, each address of the plurality being positionally distinguishable from each other address of the plurality having a unique capture probe, *e.g.*, wherein the capture probes are from a cell or subject which express 65499 or 58875 or from a cell or subject in which a 65499 or 58875 mediated response has been elicited, *e.g.*, by contact of the cell with 65499 or 58875 nucleic acid or protein, or administration to the cell or subject 65499 or 58875 nucleic acid or protein; contacting the array with one or more inquiry probe, wherein an inquiry probe can be a nucleic acid, polypeptide, or antibody (which is preferably other than 65499 or 58875 nucleic acid, polypeptide, or antibody); providing a two dimensional array having a plurality of addresses, each address of the plurality being positionally distinguishable from each other address of the plurality, and each address of the plurality having a unique capture probe, *e.g.*, wherein the capture probes are from a cell or subject which does not express 65499 or 58875 (or does not express as highly as in the case of the 65499 or 58875 positive plurality of capture probes) or from a cell or subject which in which a 65499 or 58875 mediated response has not been elicited (or has been elicited to a lesser extent than in the first sample); contacting the array with one or more inquiry probes (which is preferably other than a 65499 or 58875 nucleic acid, polypeptide, or antibody), and thereby evaluating the plurality of capture probes. Binding, *e.g.*, in the case of a nucleic acid, hybridization with a capture probe at an address of the plurality, is detected, *e.g.*, by signal generated from a label attached to the nucleic acid, polypeptide, or antibody.

In another aspect, the invention features, a method of analyzing 65499 or 58875, *e.g.*, analyzing structure, function, or relatedness to other nucleic acid or amino acid sequences. The method includes: providing a 65499 or 58875 nucleic acid or amino acid sequence, *e.g.*, a nucleotide sequence from 1-1527 of SEQ ID NO:5, or from 1-1023 of SEQ ID NO: 6, or a portion thereof; comparing the 65499 or 58875 sequence with one or more preferably a plurality of sequences from a collection of sequences, *e.g.*, a nucleic acid or protein sequence database; to thereby analyze 65499 or 58875.

The method can include evaluating the sequence identity between a 65499 or 58875 sequence and a database sequence. The method can be performed by accessing the

database at a second site, *e.g.*, over the internet. Preferred databases include GenBank™ and SwissProt.

In another aspect, the invention features, a set of oligonucleotides, useful, *e.g.*, for identifying SNP's, or identifying specific alleles of 65499 or 58875. The set includes a plurality of oligonucleotides, each of which has a different nucleotide at an interrogation position, *e.g.*, an SNP or the site of a mutation. In a preferred embodiment, the oligonucleotides of the plurality identical in sequence with one another (except for differences in length). The oligonucleotides can be provided with differential labels, such that an oligonucleotides which hybridizes to one allele provides a signal that is distinguishable from an oligonucleotides which hybridizes to a second allele.

The sequences of 65499 or 58875 molecules are provided in a variety of mediums to facilitate use thereof. A sequence can be provided as a manufacture, other than an isolated nucleic acid or amino acid molecule, which contains a 65499 or 58875 molecule. Such a manufacture can provide a nucleotide or amino acid sequence, *e.g.*, an open reading frame, in a form which allows examination of the manufacture using means not directly applicable to examining the nucleotide or amino acid sequences, or a subset thereof, as they exist in nature or in purified form.

A 65499 or 58875 nucleotide or amino acid sequence can be recorded on computer readable media. As used herein, "computer readable media" refers to any medium that can be read and accessed directly by a computer. Such media include, but are not limited to: magnetic storage media, such as floppy discs, hard disc storage medium, and magnetic tape; optical storage media such as compact disc and CD-ROM; electrical storage media such as RAM, ROM, EPROM, EEPROM, and the like; and general hard disks and hybrids of these categories such as magnetic/optical storage media. The medium is adapted or configured for having thereon 65499 or 58875 sequence information of the present invention.

As used herein, the term "electronic apparatus" is intended to include any suitable computing or processing apparatus of other device configured or adapted for storing data or information. Examples of electronic apparatus suitable for use with the present invention include stand-alone computing apparatus; networks, including a local area network (LAN), a wide area network (WAN) Internet, Intranet, and Extranet; electronic appliances such as personal digital assistants (PDAs), cellular phones, pagers, and the like; and local and distributed processing systems.

As used herein, "recorded" refers to a process for storing or encoding information on the electronic apparatus readable medium. Those skilled in the art can readily adopt any of the presently known methods for recording information on known media to generate manufactures comprising the 65499 or 58875 sequence information.

5 A variety of data storage structures are available to a skilled artisan for creating a computer readable medium having recorded thereon a 65499 or 58875 nucleotide or amino acid sequence of the present invention. The choice of the data storage structure will generally be based on the means chosen to access the stored information. In addition, a variety of data processor programs and formats can be used to store the nucleotide
10 sequence information of the present invention on computer readable medium. The sequence information can be represented in a word processing text file, formatted in commercially-available software such as WordPerfect and Microsoft Word, or represented in the form of an ASCII file, stored in a database application, such as DB2, Sybase, Oracle, or the like. The skilled artisan can readily adapt any number of data processor structuring
15 formats (*e.g.*, text file or database) in order to obtain computer readable medium having recorded thereon the nucleotide sequence information of the present invention.

By providing the 65499 or 58875 nucleotide or amino acid sequences of the invention in computer readable form, the skilled artisan can routinely access the sequence information for a variety of purposes. For example, one skilled in the art can use the
20 nucleotide or amino acid sequences of the invention in computer readable form to compare a target sequence or target structural motif with the sequence information stored within the data storage means. A search is used to identify fragments or regions of the sequences of the invention which match a particular target sequence or target motif.

The present invention therefore provides a medium for holding instructions for performing
25 a method for determining whether a subject has a seven transmembrane receptor-associated or another 65499 or 58875 -associated disease or disorder or a pre-disposition to a 65499 or 58875 -associated or another 65499 or 58875 -associated disease or disorder, wherein the method comprises the steps of determining 65499 or 58875 sequence information associated with the subject and based on the 65499 or 58875 sequence information,
30 determining whether the subject has a seven transmembrane receptor-associated or another 65499 or 58875 -associated disease or disorder and/or recommending a particular treatment for the disease, disorder, or pre-disease condition.

The present invention further provides in an electronic system and/or in a network, a method for determining whether a subject has a seven transmembrane receptor-associated or another 65499 or 58875 -associated disease or disorder or a pre-disposition to a disease associated with 65499 or 58875 , wherein the method comprises the steps of determining
5 65499 or 58875 sequence information associated with the subject, and based on the 65499 or 58875 sequence information, determining whether the subject has a seven transmembrane receptor-associated or another 65499 or 58875 -associated disease or disorder or a pre-disposition to a seven transmembrane receptor-associated or another 65499 or 58875 -associated disease or disorder, and/or recommending a particular
10 treatment for the disease, disorder, or pre-disease condition. The method may further comprise the step of receiving phenotypic information associated with the subject and/or acquiring from a network phenotypic information associated with the subject.

The present invention also provides in a network, a method for determining whether a subject has a seven transmembrane receptor-associated or another 65499 or 58875 -
15 associated disease or disorder or a pre-disposition to a seven transmembrane receptor-associated or another 65499 or 58875 -associated disease or disorder, said method comprising the steps of receiving 65499 or 58875 sequence information from the subject and/or information related thereto, receiving phenotypic information associated with the subject, acquiring information from the network corresponding to 65499 or 58875 and/or
20 corresponding to a seven transmembrane receptor-associated or another 65499 or 58875 -associated disease or disorder, and based on one or more of the phenotypic information, the 65499 or 58875 information (*e.g.*, sequence information and/or information related thereto), and the acquired information, determining whether the subject has a seven transmembrane receptor-associated or another 65499 or 58875 -associated disease or
25 disorder or a pre-disposition to a seven transmembrane receptor-associated or another 65499 or 58875 -associated disease or disorder. The method may further comprise the step of recommending a particular treatment for the disease, disorder, or pre-disease condition.

The present invention also provides a business method for determining whether a subject has a seven transmembrane receptor-associated or another 65499 or 58875 -
30 associated disease or disorder or a pre-disposition to a seven transmembrane receptor-associated or another 65499 or 58875 -associated disease or disorder, said method comprising the steps of receiving information related to 65499 or 58875 (*e.g.*, sequence information and/or information related thereto), receiving phenotypic information

associated with the subject, acquiring information from the network related to 65499 or 58875 and/or related to a seven transmembrane receptor-associated or another 65499 or 58875 -associated disease or disorder, and based on one or more of the phenotypic information, the 65499 or 58875 information, and the acquired information, determining
5 whether the subject has a seven transmembrane receptor-associated or another 65499 or 58875 -associated disease or disorder or a pre-disposition to a seven transmembrane receptor-associated or another 65499 or 58875 -associated disease or disorder. The method may further comprise the step of recommending a particular treatment for the disease, disorder, or pre-disease condition.

10 The invention also includes an array comprising a 65499 or 58875 sequence of the present invention. The array can be used to assay expression of one or more genes in the array. In one embodiment, the array can be used to assay gene expression in a tissue to ascertain tissue specificity of genes in the array. In this manner, up to about 7600 genes can be simultaneously assayed for expression, one of which can be 65499 or 58875 . This
15 allows a profile to be developed showing a battery of genes specifically expressed in one or more tissues.

In addition to such qualitative information, the invention allows the quantitation of gene expression. Thus, not only tissue specificity, but also the level of expression of a battery of genes in the tissue is ascertainable. Thus, genes can be grouped on the basis of
20 their tissue expression *per se* and level of expression in that tissue. This is useful, for example, in ascertaining the relationship of gene expression in that tissue. Thus, one tissue can be perturbed and the effect on gene expression in a second tissue can be determined. In this context, the effect of one cell type on another cell type in response to a biological stimulus can be determined. In this context, the effect of one cell type on another cell type
25 in response to a biological stimulus can be determined. Such a determination is useful, for example, to know the effect of cell-cell interaction at the level of gene expression. If an agent is administered therapeutically to treat one cell type but has an undesirable effect on another cell type, the invention provides an assay to determine the molecular basis of the undesirable effect and thus provides the opportunity to co-administer a counteracting agent
30 or otherwise treat the undesired effect. Similarly, even within a single cell type, undesirable biological effects can be determined at the molecular level. Thus, the effects of an agent on expression of other than the target gene can be ascertained and counteracted.

In another embodiment, the array can be used to monitor the time course of expression of one or more genes in the array. This can occur in various biological contexts, as disclosed herein, for example development of a seven transmembrane receptor-associated or another 65499 or 58875 -associated disease or disorder, progression of seven
5 transmembrane receptor-associated or another 65499 or 58875 -associated disease or disorder, and processes, such a cellular transformation associated with the seven transmembrane receptor-associated or another 65499 or 58875 -associated disease or disorder.

The array is also useful for ascertaining the effect of the expression of a gene on the
10 expression of other genes in the same cell or in different cells (*e.g.*, ascertaining the effect of 65499 or 58875 expression on the expression of other genes). This provides, for example, for a selection of alternate molecular targets for therapeutic intervention if the ultimate or downstream target cannot be regulated.

The array is also useful for ascertaining differential expression patterns of one or
15 more genes in normal and abnormal cells. This provides a battery of genes (*e.g.*, including 65499 or 58875) that could serve as a molecular target for diagnosis or therapeutic intervention.

As used herein, a "target sequence" can be any DNA or amino acid sequence of six or more nucleotides or two or more amino acids. A skilled artisan can readily recognize
20 that the longer a target sequence is, the less likely a target sequence will be present as a random occurrence in the database. Typical sequence lengths of a target sequence are from about 10 to 100 amino acids or from about 30 to 300 nucleotide residues. However, it is well recognized that commercially important fragments, such as sequence fragments involved in gene expression and protein processing, may be of shorter length.

Computer software is publicly available which allows a skilled artisan to access
25 sequence information provided in a computer readable medium for analysis and comparison to other sequences. A variety of known algorithms are disclosed publicly and a variety of commercially available software for conducting search means are and can be used in the computer-based systems of the present invention. Examples of such software
30 include, but are not limited to, MacPattern (EMBL), BLASTN and BLASTX (NCBI). Thus, the invention features a method of making a computer readable record of a sequence of a 65499 or 58875 sequence which includes recording the sequence on a computer readable matrix. In a preferred embodiment the record includes one or more of the

following: identification of an ORF; identification of a domain, region, or site; identification of the start of transcription; identification of the transcription terminator; the full length amino acid sequence of the protein, or a mature form thereof; the 5' end of the translated region.

5 In another aspect, the invention features a method of analyzing a sequence. The method includes: providing a 65499 or 58875 sequence, or record, in computer readable form; comparing a second sequence to the 65499 or 58875 sequence; thereby analyzing a sequence. Comparison can include comparing to sequences for sequence identity or determining if one sequence is included within the other, *e.g.*, determining if the 65499 or
10 58875 sequence includes a sequence being compared. In a preferred embodiment the 65499 or 58875 or second sequence is stored on a first computer, *e.g.*, at a first site and the comparison is performed, read, or recorded on a second computer, *e.g.*, at a second site. *E.g.*, the 65499 or 58875 or second sequence can be stored in a public or proprietary database in one computer, and the results of the comparison performed, read, or recorded
15 on a second computer. In a preferred embodiment the record includes one or more of the following: identification of an ORF; identification of a domain, region, or site; identification of the start of transcription; identification of the transcription terminator; the full length amino acid sequence of the protein, or a mature form thereof; the 5' end of the translated region.

20 This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application are incorporated herein by reference.

EXAMPLES

25

Example 1: Identification and Characterization of Human 65499 or 58875 cDNAs

The human 65499 sequence (Figure 1A-B; SEQ ID NO:1), which is approximately 1704 nucleotides long including untranslated regions, contains a predicted methionine-initiated coding sequence of about 1527 nucleotides (nucleotides 84-1610 of SEQ ID NO:1; nucleotides 1-1527 of SEQ ID NO:5), including termination codon. The coding sequence
30 encodes a 508 amino acid protein (SEQ ID NO:2).

The human 58875 sequence (Figure 8; SEQ ID NO:3), which is approximately 1278 nucleotides long including untranslated regions, contains a predicted methionine-initiated

coding sequence of about 1023 nucleotides (nucleotides 148-1170 of SEQ ID NO:3; nucleotides 1-1023 of SEQ ID NO:6, including termination codon). The coding sequence encodes a 340 amino acid protein (SEQ ID NO:4).

5 Example 2: Tissue Distribution of 65499 and 58875 mRNA

Northern blot hybridizations with various RNA samples can be performed under standard conditions and washed under stringent conditions, i.e., 0.2xSSC at 65°C. A DNA probe corresponding to all or a portion of the 65499 or 58875 cDNA (SEQ ID NO:1 or 3) can be used. The DNA was radioactively labeled with ³²P-dCTP using the Prime-It Kit
10 (Stratagene, La Jolla, CA) according to the instructions of the supplier. Filters containing mRNA from mouse hematopoietic and endocrine tissues, and cancer cell lines (Clontech, Palo Alto, CA) can be probed in ExpressHyb hybridization solution (Clontech) and washed at high stringency according to manufacturer's recommendations.

15 Example 3: Gene Expression Analysis

Total RNA was prepared from various human tissues by a single step extraction method using RNA STAT-60 according to the manufacturer's instructions (TelTest, Inc). Each RNA preparation was treated with DNase I (Ambion) at 37°C for 1 hour. DNase I treatment was determined to be complete if the sample required at least 38 PCR
20 amplification cycles to reach a threshold level of fluorescence using β -2 microglobulin as an internal amplicon reference. The integrity of the RNA samples following DNase I treatment was confirmed by agarose gel electrophoresis and ethidium bromide staining. After phenol extraction cDNA was prepared from the sample using the SUPERSRIPT™ Choice System following the manufacturer's instructions (GibcoBRL). A negative control
25 of RNA without reverse transcriptase was mock reverse transcribed for each RNA sample.

Human 58875 expression was measured by TaqMan® quantitative PCR (Perkin Elmer Applied Biosystems) in cDNA prepared from a variety of normal and diseased (e.g., cancerous) human tissues or cell lines.

Probes were designed by PrimerExpress software (PE Biosystems) based on the
30 sequence of the human 58875 gene. Each human 58875 gene probe was labeled using FAM (6-carboxyfluorescein), and the β 2-microglobulin reference probe was labeled with a different fluorescent dye, VIC. The differential labeling of the target gene and internal

reference gene thus enabled measurement in same well. Forward and reverse primers and the probes for both β 2-microglobulin and target gene were added to the TaqMan® Universal PCR Master Mix (PE Applied Biosystems). Although the final concentration of primer and probe could vary, each was internally consistent within a given experiment. A typical experiment contained 200nM of forward and reverse primers plus 100nM probe for β -2 microglobulin and 600 nM forward and reverse primers plus 200 nM probe for the target gene. TaqMan matrix experiments were carried out on an ABI PRISM 7700 Sequence Detection System (PE Applied Biosystems). The thermal cycler conditions were as follows: hold for 2 min at 50°C and 10 min at 95°C, followed by two-step PCR for 40 cycles of 95°C for 15 sec followed by 60°C for 1 min.

The following method was used to quantitatively calculate human 58875 gene expression in the various tissues relative to β -2 microglobulin expression in the same tissue. The threshold cycle (Ct) value is defined as the cycle at which a statistically significant increase in fluorescence is detected. A lower Ct value is indicative of a higher mRNA concentration. The Ct value of the human 58875 gene is normalized by subtracting the Ct value of the β -2 microglobulin gene to obtain a Δ Ct value using the following formula: Δ Ct = Ct_{human 59914 and 59921} - Ct _{β -2 microglobulin}. Expression is then calibrated against a cDNA sample showing a comparatively low level of expression of the human 58875 gene. The Δ Ct value for the calibrator sample is then subtracted from Δ Ct for each tissue sample according to the following formula: $\Delta\Delta$ Ct = Δ Ct_{sample} - Δ Ct_{calibrator}. Relative expression is then calculated using the arithmetic formula given by $2^{-\Delta\Delta$ Ct}. Expression of the target human 58875 gene in each of the tissues tested is then graphically represented as discussed in more detail below.

TaqMan real-time quantitative RT-PCR is used to detect the presence of RNA transcript corresponding to human 58875 relative to a no template control in a panel of human tissues or cells. It is found that the highest expression of 58875 orthologs are expressed in normal brain cortex tissue, as shown in table 1. There is also relatively minute expression seen in brain hypothalamus tissue.

Table 1

Tissue Type	Mean	β 2 Mean	$\Delta\Delta$ Ct	Expression
Artery normal	40	20.04	19.97	0
Aorta diseased	40	22.55	17.45	0

	Vein normal	40	19.57	20.43	0
	Coronary SMC	40	20.08	19.92	0
	HUVEC	40	20.38	19.62	0
	Hemangioma	40	19.41	20.59	0
5	Heart normal	40	19.95	20.06	0
	Heart CHF	40	20.54	19.46	0
	Kidney	40	20.27	19.73	0
	Skeletal Muscle	40	21.55	18.45	0
	Liver normal	40	19.27	20.73	0
10	Small intestine normal	36.55	19.87	16.68	0
	Adipose normal	40	19.14	20.86	0
	Pancreas	40	21.68	18.32	0
	primary osteoblasts	40	19.48	20.52	0
	Bladder-Female normal	39.91	19.43	20.48	0
15	Adrenal Gland normal	40	18.96	21.04	0
	Pituitary Gland normal	40	19.56	20.45	0
	Spinal cord normal	39.3	20.36	18.95	0
	Brain Cortex normal	27.91	22.19	5.71	19.0377
	Brain Hypothalamus normal	32	20.42	11.58	0.3266
20	Nerve	40	20.19	19.81	0
	DRG (Dorsal Root Ganglion)	40	20.68	19.32	0
	Breast normal	40	20.44	19.56	0
	Breast tumor/IDC	40	19.27	20.73	0
	Ovary normal	40	19.83	20.17	0
25	Ovary Tumor	40	19.14	20.86	0
	Prostate BPH	40	19.3	20.7	0
	Prostate Adenocarcinoma	40	20.07	19.93	0
	Colon normal	40	19.11	20.89	0
	Colon Adenocarcinoma	40	21.25	18.75	0
30	Lung normal	40	18.03	21.97	0
	Lung tumor	40	20.23	19.77	0
	Lung COPD	40	18.44	21.56	0
	Colon IBD	40	19.54	20.47	0

	Synovium	40	19	21	0
	Tonsil normal	40	18.19	21.81	0
	Lymph node normal	40	19.86	20.14	0
	Liver fibrosis	40	20.47	19.53	0
5	Spleen normal	40	18.35	21.65	0
	Macrophages	40	16.67	23.33	0
	Progenitors (erythroid, megakaryocyte, neutrophil)				
		40	19.29	20.72	0
	Megakaryocytes	39.58	18.91	20.68	0
10	Activated PBMC	39.44	16.48	22.95	0
	Neutrophils	40	18.3	21.7	0
	Erythroid	40	20.84	19.16	0
	positive control	27.7	21.07	6.64	10.0268

15 Example 4: Recombinant Expression of 65499 or 58875 in Bacterial Cells

In this example, 65499 or 58875 is expressed as a recombinant glutathione-S-transferase (GST) fusion polypeptide in *E. coli* and the fusion polypeptide is isolated and characterized. Specifically, 65499 or 58875 is fused to GST and this fusion polypeptide is expressed in *E. coli*, e.g., strain PEB199. Expression of the GST-65499 or GST-58875 fusion protein in PEB199 is induced with IPTG. The recombinant fusion polypeptide is purified from crude bacterial lysates of the induced PEB199 strain by affinity chromatography on glutathione beads. Using polyacrylamide gel electrophoretic analysis of the polypeptide purified from the bacterial lysates, the molecular weight of the resultant fusion polypeptide is determined.

25

Example 5: Expression of Recombinant 65499 or 58875 Protein in COS Cells

To express the 65499 or 58875 gene in COS cells, the pcDNA/Amp vector by Invitrogen Corporation (San Diego, CA) is used. This vector contains an SV40 origin of replication, an ampicillin resistance gene, an *E. coli* replication origin, a CMV promoter followed by a polylinker region, and an SV40 intron and polyadenylation site. A DNA fragment encoding the entire 65499 or 58875 protein and an HA tag (Wilson et al. (1984) *Cell* 37:767) or a FLAG tag fused in-frame to its 3' end of the fragment is cloned into the

polylinker region of the vector, thereby placing the expression of the recombinant protein under the control of the CMV promoter.

To construct the plasmid, the 65499 or 58875 DNA sequence is amplified by PCR using two primers. The 5' primer contains the restriction site of interest followed by approximately twenty nucleotides of the 65499 or 58875 coding sequence starting from the initiation codon; the 3' end sequence contains complementary sequences to the other restriction site of interest, a translation stop codon, the HA tag or FLAG tag and the last 20 nucleotides of the 65499 or 58875 coding sequence. The PCR amplified fragment and the pCDNA/Amp vector are digested with the appropriate restriction enzymes and the vector is dephosphorylated using the CIAP enzyme (New England Biolabs, Beverly, MA). Preferably the two restriction sites chosen are different so that the 65499 or 58875 gene is inserted in the correct orientation. The ligation mixture is transformed into *E. coli* cells (strains HB101, DH5 α , SURE, available from Stratagene Cloning Systems, La Jolla, CA, can be used), the transformed culture is plated on ampicillin media plates, and resistant colonies are selected. Plasmid DNA is isolated from transformants and examined by restriction analysis for the presence of the correct fragment.

COS cells are subsequently transfected with the 65499- or 58875-pcDNA/Amp plasmid DNA using the calcium phosphate or calcium chloride co-precipitation methods, DEAE-dextran-mediated transfection, lipofection, or electroporation. Other suitable methods for transfecting host cells can be found in Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual*. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989. The expression of the 65499 or 58875 polypeptide is detected by radiolabelling (^{35}S -methionine or ^{35}S -cysteine available from NEN, Boston, MA, can be used) and immunoprecipitation (Harlow, E. and Lane, D. *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1988) using an HA specific monoclonal antibody. Briefly, the cells are labeled for 8 hours with ^{35}S -methionine (or ^{35}S -cysteine). The culture media are then collected and the cells are lysed using detergents (RIPA buffer, 150 mM NaCl, 1% NP-40, 0.1% SDS, 0.5% DOC, 50 mM Tris, pH 7.5). Both the cell lysate and the culture media are precipitated with an HA specific monoclonal antibody. Precipitated polypeptides are then analyzed by SDS-PAGE.

Alternatively, DNA containing the 65499 or 58875 coding sequence is cloned directly into the polylinker of the pCDNA/Amp vector using the appropriate restriction sites. The resulting plasmid is transfected into COS cells in the manner described above, and the expression of the 65499 or 58875 polypeptide is detected by radiolabelling and
5 immunoprecipitation using a 65499 or 58875 specific monoclonal antibody.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention
10 described herein. Such equivalents are intended to be encompassed by the following claims.

What is claimed is:

1. An isolated 65499 or 58875 nucleic acid molecule selected from the group consisting of:
 - a) a nucleic acid molecule comprising a nucleotide sequence which is at least
5 60% identical to the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____;
 - b) a nucleic acid molecule comprising a fragment of at least 15 nucleotides of the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:6, or
10 the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____;
 - c) a nucleic acid molecule which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number _____;
 - 15 d) a nucleic acid molecule which encodes a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number _____, wherein the fragment comprises at least 15 contiguous amino acids of SEQ ID NO:2, SEQ ID NO:4, or the amino acid sequence encoded by the cDNA
20 insert of the plasmid deposited with the ATCC as Accession Number _____;
 - e) a nucleic acid molecule which encodes a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:\$, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number _____, wherein the nucleic acid molecule hybridizes to a
25 nucleic acid molecule comprising SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:6, or a complement thereof, under stringent conditions;
 - f) a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____; and
 - 30 g) a nucleic acid molecule which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number _____.

2. The isolated nucleic acid molecule of claim 1, which is the nucleotide sequence SEQ ID NO:1 or SEQ ID NO:3.
3. A host cell which contains the nucleic acid molecule of claim 1.
4. An isolated 65499 or 58875 polypeptide selected from the group consisting
5 of:
 - a) a polypeptide which is encoded by a nucleic acid molecule comprising a nucleotide sequence which is at least 60% identical to a nucleic acid comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession
10 Number _____, or a complement thereof;
 - b) a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number _____, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a
15 nucleic acid molecule comprising SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:6, or a complement thereof under stringent conditions;
 - c) a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number _____, wherein the fragment
20 comprises at least 15 contiguous amino acids of SEQ ID NO:2 or SEQ ID NO:4; and
 - d) the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4.
5. An antibody which selectively binds to a polypeptide of claim 4.
6. A method for producing a polypeptide selected from the group consisting of:
 - a) a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ
25 ID NO:4, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number _____;
 - b) a polypeptide comprising a fragment of the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number _____, wherein the fragment

comprises at least 15 contiguous amino acids of SEQ ID NO:2, SEQ ID NO:4, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number _____;

5 c) a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number _____, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule comprising SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, or SEQ ID NO:6; and

10 d) the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4; comprising culturing the host cell of claim 3 under conditions in which the nucleic acid molecule is expressed.

7. A method for detecting the presence of a nucleic acid molecule of claim 1 or a polypeptide encoded by the nucleic acid molecule in a sample, comprising:

15 a) contacting the sample with a compound which selectively hybridizes to the nucleic acid molecule of claim 1 or binds to the polypeptide encoded by the nucleic acid molecule; and

b) determining whether the compound hybridizes to the nucleic acid or binds to the polypeptide in the sample.

20 8. A kit comprising a compound which selectively hybridizes to a nucleic acid molecule of claim 1 or binds to a polypeptide encoded by the nucleic acid molecule and instructions for use.

9. A method for identifying a compound which binds to a polypeptide or modulates the activity of the polypeptide of claim 4 comprising the steps of:

25 a) contacting a polypeptide, or a cell expressing a polypeptide of claim 4 with a test compound; and

b) determining whether the polypeptide binds to the test compound or determining the effect of the test compound on the activity of the polypeptide.

10. A method for modulating the activity of a polypeptide of claim 4 comprising contacting the polypeptide or a cell expressing the polypeptide with a compound which binds to the polypeptide in a sufficient concentration to modulate the activity of the polypeptide.

5 11. A method of identifying a nucleic acid molecule associated with a disorder comprising:

a) contacting a sample from a subject with or at risk of developing a disorder comprising nucleic acid molecules with a hybridization probe comprising at least 25 contiguous nucleotides of SEQ ID NO:1 or SEQ ID NO:3 defined in claim 2; and

10 b) detecting the presence of a nucleic acid molecule in the sample that hybridizes to the probe, thereby identifying a nucleic acid molecule associated with a disorder.

12. A method of identifying a nucleic acid associated with a disorder comprising:

15 a) contacting a sample from a subject having a disorder or at risk of developing a disorder comprising nucleic acid molecules with a first and a second amplification primer, the first primer comprising at least 25 contiguous nucleotides of SEQ ID NO:1 or SEQ ID NO:3 defined in claim 2 and the second primer comprising at least 25 contiguous nucleotides from the complement of SEQ ID NO:1 or SEQ ID NO:3;

20 b) incubating the sample under conditions that allow nucleic acid amplification; and

c) detecting the presence of a nucleic acid molecule in the sample that is amplified, thereby identifying the nucleic acid molecule associated with a disorder.

25 13. A method of identifying a polypeptide associated with a disorder comprising:

a) contacting a sample comprising polypeptides with a 65499 or 58875 binding partner of the 65499 or 58875 polypeptide defined in claim 4; and

b) detecting the presence of a polypeptide in the sample that binds to the 65499 or 58875 binding partner, thereby identifying the polypeptide associated with a disorder.

14. A method of identifying a subject having a disorder or at risk for developing a disorder comprising:

- a) contacting a sample obtained from the subject comprising nucleic acid molecules with a hybridization probe comprising at least 25 contiguous nucleotides of SEQ ID NO:1 or SEQ ID NO:3 defined in claim 2; and
- b) detecting the presence of a nucleic acid molecule in the sample that hybridizes to the probe, thereby identifying a subject having a disorder or at risk for developing a disorder.

15. A method of identifying a subject having a disorder or at risk for developing a disorder comprising:

- a) contacting a sample obtained from the subject comprising nucleic acid molecules with a first and a second amplification primer, the first primer comprising at least 25 contiguous nucleotides of SEQ ID NO:1 or SEQ ID NO:3 defined in claim 2 and the second primer comprising at least 25 contiguous nucleotides from the complement of SEQ ID NO:1 or SEQ ID NO:3;
- b) incubating the sample under conditions that allow nucleic acid amplification; and
- c) detecting the presence of a nucleic acid molecule in the sample that is amplified, thereby identifying a subject having a disorder or at risk for developing a disorder.

16. A method of identifying a subject having a disorder or at risk for developing a disorder comprising:

- a) contacting a sample obtained from the subject comprising polypeptides with a 65499 or 58875 binding partner of the 65499 or 58875 polypeptide defined in claim 4; and
- b) detecting the presence of a polypeptide in the sample that binds to the 65499 or 58875 binding partner, thereby identifying a subject having a disorder or at risk for developing a disorder.

17. A method for identifying a compound capable of treating a disorder characterized by aberrant 65499 or 58875 nucleic acid expression or 65499 or 58875

polypeptide activity comprising assaying the ability of the compound to modulate 65499 or 58875 nucleic acid expression or 65499 or 58875 polypeptide activity, thereby identifying a compound capable of treating a disorder characterized by aberrant 65499 or 58875 nucleic acid expression or 65499 or 58875 polypeptide activity.

- 5 18. A method for treating a subject having a disorder or at risk of developing a disorder comprising administering to the subject a 65499 or 58875 modulator of the nucleic acid molecule defined in claim 1 or the polypeptide encoded by the nucleic acid molecule or contacting a cell with a 65499 or 58875 modulator.

19. The method of claim 18, wherein the 65499 or 58875 modulator is
- 10 a) a small molecule;
- b) peptide;
- c) phosphopeptide;
- d) anti-65499 or 58875 antibody;
- e) a 65499 or 58875 polypeptide comprising the amino acid sequence of SEQ
- 15 ID NO:2, SEQ ID NO:4, or a fragment thereof;
- f) a 65499 or 58875 polypeptide comprising an amino acid sequence which is at least 90 percent identical to the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, wherein the percent identity is calculated using the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap
- 20 penalty of 4; or
- g) an isolated naturally occurring allelic variant of a polypeptide consisting of the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a complement of a nucleic acid molecule consisting of SEQ ID NO:1 at 6X SSC at 45°C, followed by one or more washes
- 25 in 0.2X SSC, 0.1% SDS at 65°C.

20. The method of claim 18, wherein the 65499 or 58875 modulator is
- a) an antisense 65499 or 58875 nucleic acid molecule;
- b) is a ribozyme;
- c) the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, or a fragment
- 30 thereof;

- d) a nucleic acid molecule encoding a polypeptide comprising an amino acid sequence which is at least 90 percent identical to the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, wherein the percent identity is calculated using the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4;
- e) a nucleic acid molecule encoding a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, wherein the nucleic acid molecule which hybridizes to a complement of a nucleic acid molecule consisting of SEQ ID NO:1 at 6X SSC at 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 65°C; or
- f) a gene therapy vector.

21. A method for evaluating the efficacy of a treatment of a disorder, in a subject, comprising:
- treating a subject with a protocol under evaluation;
- assessing the expression level of a 65499 or 58875 nucleic acid molecule defined in claim 1 or 65499 or 58875 polypeptide encoded by the 65499 or 58875 nucleic acid molecule,
- wherein a change in the expression level of 65499 or 58875 nucleic acid or 65499 or 58875 polypeptide after the treatment, relative to the level before the treatment, is indicative of the efficacy of the treatment of a disorder.

22. A method of diagnosing a disorder in a subject, comprising:
- evaluating the expression or activity of a 65499 or 58875 nucleic acid molecule defined in claim 1 or a 65499 or 58875 polypeptide encoded by the 65499 or 58875 nucleic acid molecule, such that a difference in the level of 65499 or 58875 nucleic acid or 65499 or 58875 polypeptide relative to a normal subject or a cohort of normal subjects is indicative of a disorder.

23. The method defined in claim 18, wherein the disorder is cancer or aberrant cellular proliferation and/or differentiation, disorders associated with bone metabolism, hematopoietic disorders, cardiovascular disorders, including endothelial cell disorders, blood vessel disorders, brain disorders, and hormonal disorders.

TGAGTGCCTGTGTCACCCCTTTGCCACCCACCGGTGCCCGGCACTGAGCCCTGCAACCTGTCTCAGCCCTCTCGGCTGT	SEQ ID NO:1
M T S T C T N S T R E S N S S H T C	SEQ ID NO:2
TGCC ATG ACG TCC ACC TGC ACC AAC AGC ACG CGC GAG AGT AAC AGC AGC CAC ACG TGC	51
↑SEQ ID NO:3→	
M P L S K M P I S L A H G I I R S T V L	38
ATG CCC CTC TCC AAA ATG CCC ATC AGC CTG GCC CAC GGC ATC ATC CGC TCA ACC GTG CTG	114
V I F P A A S F V G N I V L A L V L Q R	53
GTT ATC TTC CCC GCC GCC TCT TTC GTC GGC AAC ATA GTG CTG GCG CTA GTG TTG CAG CGC	174
K P Q L L Q V T N R F I F N L L V T D L	73
AAG CCG CAG CTG CTG CAG GTG ACC AAC CGT TTT ATC TTT AAC CTC CTC GTC ACC GAC CTG	234
L Q I S L V A P W V V A T S V P L F W P	38
CTG CAG ATT TCG CTC GTG GCC CCC TGG GTG GTG GCC ACC TCT GTG CCT CTC TTC TGG CCC	294
L N S H F C T A L V S P T H L F A F A S	118
CTC AAC AGC CAC TTC TGC ACG GCC CTG GTT AGC CCC ACC CAC CTG TTC GCC TTC GCC AGC	354
V N T I V L V S V D R Y L S I I H P L S	138
GTC AAC ACC ATT GTC TTG GTG TCA GTG GAT CGC TAC TTG TCC ATC ATC CAC CCT CTC TCC	414
Y P S K M T Q R R G Y L L L Y G T W I V	158
TAC CCG TCC AAG ATG ACC CAG CGC CGC GGT TAC CTG CTC CTC TAT GGC ACC TGG ATT GTG	474
A I L Q S T P P L Y G W G Q A A F D E R	178
GCC ATC CTG CAG AGC ACT CCT CCA CTC TAC GGC TGG GGC CAG GCT GCC TTT GAT GAG CGC	534
N A L C S M T W G A S P S Y T I L S V V	198
AAT GCT CTC TGC TCC ATG ACC TGG GGG GCC AGC CCC AGC TAC ACT ATT CTC AGC GTG GTG	594
S F I V I P L I V M I A C Y S V V F C A	218
TCC TTC ATC GTC ATT CCA CTG ATT GTC ATG ATT GCC TGC TAC TCC GTG GTG TTC TGT GCA	654
A R R Q H A L L Y N V K R H S L E V R V	238
GCC CGG AGG CAG CAT GCT CTG CTG TAC AAT GTC AAG AGA CAC AGC TTG GAA GTG CGA GTC	714
K D C V E N E D E E G A E K K E E F Q D	258
AAG GAC TGT GTG GAG AAT GAG GAT GAA GAG GGA GCA GAG AAG AAG GAG GAG TTC CAG GAT	774
E S E F R R Q H E G E V K A K E G R M E	278
GAG AGC GAG TTT CGC CGC CAG CAT GAA GGC GAG GTC AAG GCC AAG GAG GGC AGA ATG GAA	834
A K D G S L K A K E G S T G I S E S S V	298
GCC AAG GAC GGC AGC CTG AAG GCC AAG GAA GGA AGC ACG GGG ATC AGT GAG AGT AGT GTA	894
E A R G S E E V R E S S T V A S D G S M	318
GAG GCC AGG GGC AGC GAG GAG GTC AGA GAG AGC AGC ACG GTG GCC AGC GAC GGC AGC ATG	954
E G K E G S T K V E E N S M K A D K G R	338
GAG GGT AAG GAA GGC AGC ACC AAA GTT GAG GAG AAC AGC ATG AAG GCA GAC AAG GGT CGC	1014
T E V N Q C S I D L G E D D H E F G E D	358
ACA GAG GTC AAC CAG TGC AGC ATT GAC TTG GGT GAA GAT GAC ATG GAG TTT GGT GAA GAC	1074
D I N F S E D D V E A V N I P E S L P P	378
GAC ATC AAT TTC AGT GAG GAT GAC GTC GAG GCA GTG AAC ATC CCG GAG AGC CTC CCA CCC	1134
S R R N S N S N P P L P R C Y Q C K A A	398
AGT CGT CGT AAC AGC AAC AGC AAC CCT CCT CTG CCC AGG TGC TAC CAG TGC AAA GCT GCT	1194
K V I F I I I F S Y V L S L G P Y C F L	418
AAA GTG ATC TTC ATC ATT ATT TTC TCC TAT GTG CTA TCC CTG GGG CCC TAC TGC TTT TTA	1254
A V L A V W V D V E T Q V P Q W V I T I	438
GCA GTC CTG GCC GTG TGG GTG GAT GTC GAA ACC CAG GTA CCC CAG TGG GTG ATC ACC ATA	1314
I I W L F F L Q C C I H P Y V Y G Y M H	458

FIG. 1a

ATC ATC TGG CTT TTC TTC CTG CAG TGC TGC ATC CAC CCC TAT GTC TAT GGC TAC ATG CAC 1374
K T I K K E I Q D M L K K F F C K E K P 479
AAG ACC ATT AAG AAG GAA ATC CAG GAC ATG CTG AAG AAG TTC TTC TGC AAG GAA AAG CCC 1414
P K E D S H P D L P G T E G G T E G K I 498
CCG AAA GAA GAT AGC CAC CCA GAC CTG CCC GGA ACA GAG GGT GGG ACT GAA GGC AAG ATT 1494
V P S Y D S A T F P * 509
GTC CCT TCC TAC GAT TCT GCT ACT TTT CCT TGA 1527
←SEQ ID NO:5↑
AGTTAGTTCTAAGGCAAACCTTGAAGTGTCCATAACACGAGAAACAAGAGGAGATTCTTTTCAATGGACCCACAATT
ATTAATGCCAAACCA

FIG. 1b

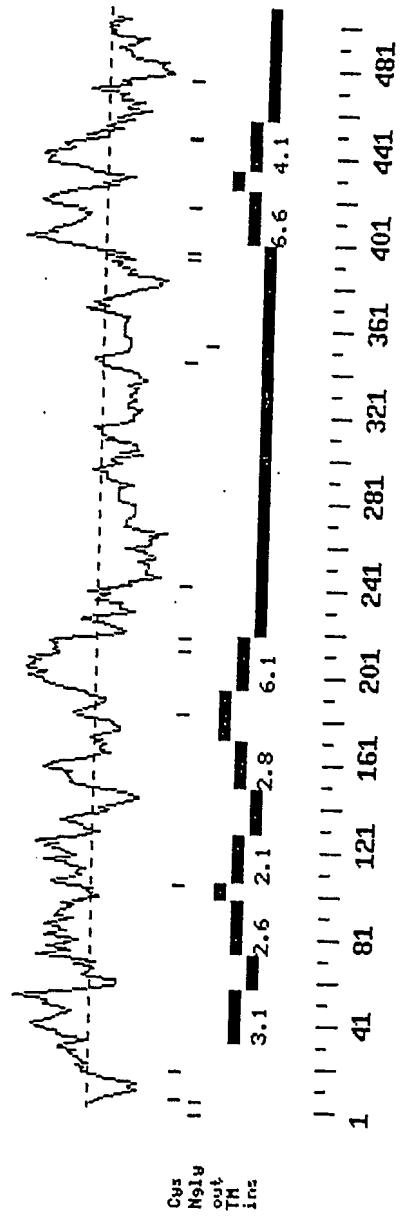


FIG. 2

```

*->GNLLVilviltklrtptnifilNLAvADLLflltclppwalyylvg  SEQ ID NO:7
GN++ lv+ r +l +tn fi+NL v+DLL ++ pw++ ++v
65499 48 GNIVLALVLQRKPQLLQVTNRFI FNLLVTDLLQISLVAPWVWVATSVP 94

gsedWpfGsalCklvtaldvvnmyaSi111LtaISiDRYLAIvhlPrYrrr
Wp+ s++C +++ ++ +aS+ ++++ S+DRYL+I+hPl+y+++
65499 95 L--FWPLNSHFCTALVSPTHLFAFASVNTIVLVSVDRYLSIIHPLSYPSK 142

rtsprrrAkvvillvWvlalllslPpllfswvktveegngtlnvntvCli
+t rr+++++ ++W++a+l s Ppl+++++ + e+ C++
65499 143 MT-QRRGYLLLYGTWIVAILQSTPPLYGWGQAAFDER-----NALCSM 184

dfpeestasvstwlrsyvl1stlvgFl1Pl1vilvcYtr1lrtl....
++ ++ sy++ls + + ++Pl+v++ cY++++++r+++
65499 185 TWGASP-----SYTILSVVSFIVIPLIVMIACYSVVFCAARqhal 225

.....
+ +++++ + + ++ ++++++ ++++++ ++++++ ++++++ + +
65499 226 lynvkrhslevrvkdcvenedeegaekkeefqdesefrrqhegevkakeg 275

.....
+ +++++ + ++++++ ++++++ ++++++ ++++++ ++++++ ++++++
65499 276 rmeakdgs1kakegstgisessveargseevresstvasdgsmegkegst 325

.....
+ +++++ + ++++++ ++ + +++++ ++++++ ++++++ + + +
65499 326 kveensmkadkgrtevnqcsidlgeddmeffgeddinfseddveavnipes 375

.....kaaktllvvvvvFv1CW1Pyfiv1lldtlc
+++++ ++++++ ++ + kaak+++++ vl+ Py +++l+++
65499 376 lpsrrnsnsnpplprcyqcKAAKVFIIIFSVYLSLGPYCF1AVLAVWV 425

.lsiimsstCelervlptallvtlwLayvNsc1NPi1Y<-*
+ ++++++ ++++++ ++ +c+ P +Y
65499 426 dVE-----TQVPQWVITIIIWLF1LQCCIHPYVY 454

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FIG. 3

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Query: 61 QLLQVT-NRFIFNLLVTDLLQISLVAPWVWVATSVP-----LFW-PLNSHFCTALVSPTH 112
+L VT N F+ NL+ DLL L P+++ S+ FW S C L S +
Sbjct: 4 KLRTVTMNYFLLNLAIADLLVAILTLFPMIVYSLTNFYSQWYFWSIFGSC1CK-LYSFLY 62 SEQ ID NO:8

Query: 113 LFA-FASVNTIVLVSVDRYLSIIHP-----LSYPSKMTQRRGYLLLYGTWIVAILQST 164
+ + +AS+ T+ ++S+DRYL+I HP L Y + MT+RR L++ W+++IL S
Sbjct: 63 VLSMYASIFTTLVISIDRYLAICHPMMSYKRLHYTTIMTKRRAVLVIAVWVLSILISL 122

Query: 165 PPLYGW 170
PPL W
Sbjct: 123 PPLIFW 128

```

FIG. 4

Query: 394 QCKAAKVIFIIIFSIVLSLGPYCF-LAVLAVWVDVETQVPQWVITIIWLFFLQCCIHYPY 452
 QCKA I +++ +++++ GPY +A A+W ++ V + T WL F HP
 Sbjct: 99 QCKALITILVVLGAFMVTWGPYMVVIASEALWG--KSSVSPSLETWATWLSFASAVCHPL 156 SEQ ID NO:9

Query: 453 VYGYMHKTIKKEIQDM 468
 +YG +KT++KE+ M
 Sbjct: 157 IYGLWNKTVRKELLGM 172

FIG. 5a

Query: 175 FDERNALCSMTWGASPSYTIILSVVSFIVIPLIVMIACYSVVFCAAR 220
 FDE +C W P YT + + P +VM+ CY +F AR
 Sbjct: 5 FDEFKWMCVAAWHREPGYTAFWQIWICALFPFLVMLVCYGFIFRVAR 50 SEQ ID NO:10

FIG. 5b

Query: 170 WQQAEDERNALCSMT---WGASPSYTIILSVVSFIVIPLIVMIACYSVVFCAARRQHALL 226
 WG+ DE CS+ G SP T+ +V F+ IP +V+I CY+ +F Q +
 Sbjct: 2 WGEFGLDEATFSCSIMKDEHGRSPKKTIF-IVGFM-IPCLVIIVCYACIFLTVHHQKKKI 59 SEQ ID NO:11

Query: 227 YN 228
 N
 Sbjct: 60 RN 61

FIG. 6a

Query: 396 KAAKVIFIIIFSIVLSLGPYCFLAVLAVWVDV-----ETQVPQWVITIIWLFFLQCCIH 450
 K K++ ++ + + P L VL + + + W WL + CI+
 Sbjct: 95 KTKMLMVVFLCFAICYLPISILNVLKRVFGRHSEDNESVYWWHIFSHWLVIYANSCIN 154 SEQ ID NO:12

Query: 451 PYVYGYMHKTIKK 463
 P +Y +M+ +K
 Sbjct: 155 PIIYNFMNGKYRK 167

FIG. 6b

Query: 28 LAHGII---RSTVLVIFPAASFVGNIVLALVLQKPKQLLQVTNRFI FNLLVTDLLQISLV 84
L+HG ++ VL++ S +GN + V+ + QL VTN FI +L ++DLL L
Sbjct: 85 LSHGA AVAAQALVLLIFLLSSLGNCAVMGVIVKHRQLRTVTNAFILSLSLSDLLTALLC 144 SEQ ID NO:13

Query: 85 APWVVATSVPLFWPLNSHFCTALVSPTHLFAFAS---VNTIVLVSVDRYLSIIHPL-SY- 139
P A + LF P P F S + +V R ++ PL Y
Sbjct: 145 LP---AAFLDLFTPPGGSAPALPAGPWRGFCRPSRFFSSCFGIVYAQRGAHLVGPLLR YR 201

Query: 140 -P--SKMTQRRGYLLLYGTWIVAILQSTP-PLYGWG-QAAFDERNALCSMTWGASPS--- 191
P K+ +RR LL G W+ A+ S P L G + A + C P+
Sbjct: 202 RPPREKIGRRRALQLLAGAWLTALGFSLPWELLGAPRELAAGQSFHGCLYRTSPDPAQLG 261

Query: 192 --YTILSVVSFIVIPLIVMIACY 212
+++ VV+ ++P +++ C+
Sbjct: 262 GPFSVGLVVACYLLPFLLICFCH 284

FIG. 7

AGCACGAGGGAAGAGCAGCAGCACCCGATGCTCGGATTGGATGAAGTGCAAGGTAACCCCGCTGGTTCGGGCGT SEQ ID NO:3

TTGGCTGTTCCTGTGCCTGGACCCCGCTTTGGAAATGGAGCTTTAATCCCTGGAAAGTCCACGAACA M N SEQ ID NO:4
 ↑SEQ ID NO:6→ ATG AAT

P F H A S C W N T S A E L L N K S W N K 22
 CCA TTT CAT GCA TCT TGT TGG AAC ACC TCT GCC GAA CTT TTA AAC AAA TCC TGG AAT AAA 66

E F A Y Q T A S V V D T V I L P S M I G 42
 GAG TTT GCT TAT CAA ACT GCC AGT GTG GTA GAT ACA GTC ATC CTC CCT TCC ATG ATT GGG 126

I I C S T G L V G N I L I V F T I I R S 52
 ATT ATC TGT TCA ACA GGG CTG GTT GGC AAC ATC CTC ATT GTA TTC ACT ATA ATA AGA TCC 186

R K K T V P D I Y T C N L A V A D L V H 82
 AGG AAA AAA ACA GTC GAC ATC TAT ACC TGC AAC CTG GCT GTG GCT GAT TTG GTC CAC 146

I V G M P F L I H Q W A R G G E W V F G 102
 ATA GTT GGA ATG CCT TTT CTT ATT CAC CAA TGG GCC CGA GGG GGA GAG TGG GTG TTT GGG 166

G P L C T I I T S L D T C N Q F A C S A 122
 GGG CCT CTC TGC ACC ATC ATC ACA TCC CTG GAT ACT TGT AAC CAA TTT GCC TGT AGT GCC 186

I M T V M S V D R Y F A L V Q P F R L T 142
 ATC ATG ACT GTA ATG AGT GTG GAC AGG TAC TTT GCC CTC GTC CAA CCA TTT CGA CTG ACA 206

R W R T R Y K T I R I N L G L W A A S F 162
 CGT TGG AGA ACA AGG TAC AAG ACC ATC CGG ATC AAT TTG GGC CTT TGG GCA GCT TCC TTT 226

I L A L P V W V Y S K V I K F K D G V E 182
 ATC CTG GCA TTG CCT GTC TGG GTC TAC TCG AAG GTC ATC AAA TTT AAA GAC GGT GTT GAG 246

S C A F D L T S P D D V L W Y T L Y L T 202
 AGT TGT GCT TTT GAT TTG ACA TCC CCT GAC GAT GTA CTC TGG TAT ACA CTT TAT TTG ACG 266

I T T F F F P L P L I L V C Y I L I L C 222
 ATA ACA ACT TTT TTT TTC CCT CTA CCC TTG ATT TTG GTG TGC TAT ATT TTA ATT TTA TGC 286

Y T W E M Y Q Q N K D A R C C N P S V P 242
 TAT ACT TGG GAG ATG TAT CAA CAG AAT AAG GAT GCC AGA TGC TGC AAT CCC AGT GTA CCA 306

K Q R V M K L T K M V L V L V V V F I L 262
 AAA CAG AGA GTG ATG AAG TTG ACA AAG ATG GTG CTG GTG CTG GTG GTG GTC TTT ATC CTG 366

S A A P Y H V I Q L V N L Q M E Q P T L 282
 AGT GCT GCC CCT TAT CAT GTG ATA CAA CTG GTG AAC TTA CAG ATG GAA CAG CCC ACA CTG 346

A F Y V G Y Y L S I C L S Y A S S S I N 302
 GCC TTC TAT GTG GGT TAT TAC CTC TCC ATC TGT CTC AGC TAT GCC AGC AGC AGC ATT AAC 366

P F L Y I L L S G N F Q K R L P Q I Q R 322
 CCT TTT CTC TAC ATC CTG CTG AGT GGA AAT TTC CAG AAA CGT CTG CCT CAA ATC CAA AGA 386

R A T E K E I N N M G N T L K S H F * 341
 AGA GCG ACT GAG AAG GAA ATC AAC AAT ATG GGA AAC ACT CTG AAA TCA CAC TTT TAG 401
 ←SEQ ID NO:6→

GAAAGTACATGGATCACCATGAGTCTAGACATGATTGTCTATCTTACTGGTATTATTAGAAAGGCCGGTGTACCGATA

TGTTTATGCCCATTTCTTCTGTGTACTTG

FIG. 8

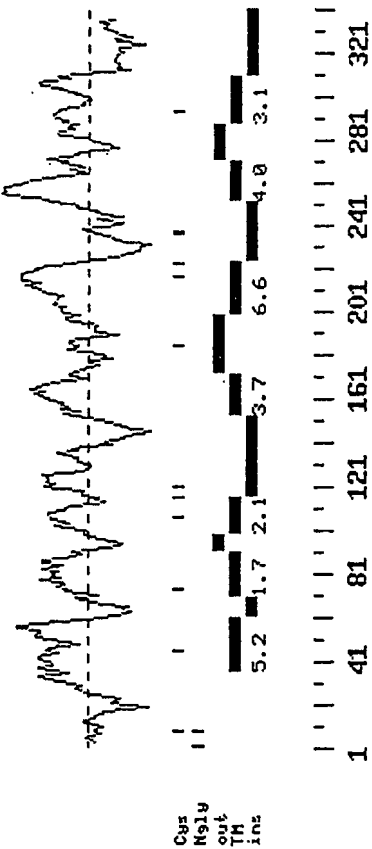


FIG. 9

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*->GNLVilviltkklrtptnifiNLAvADLLflltppwalyylvg SEQ ID NO:14
GN+L++++i+r + t++++i+ +NLAvADL + p+ + ++ g
58875 51 GNILIVFTIIR-SRKKTVDPDIYTCNLAVADLVHIVGMPFLIHQWARG 96

gsedWpfGsalCklvtaldvvnmyaSi11LtaISiDRYlAivhPlryrrr
g +W+fG lC + t+ld++n +a +t++S+DRY A v P+r r+
58875 97 G--EWWFGGPLCTIITS1DTCNQFACSAIMTVMSVDRYFALVQPFRLTRW 144

rtsprrrAkvvillvWvlal11slPpl1fswvktveegngtlnvntvCli
rt + ++ + l+ W+++++l+1P ++s+v + +g v +C +
58875 145 RT-RYKTIRINLGLWAASFILALPVWVYSKVIKFKDG-----VESCAF 186

dfpeestasvstwlrsyvl1stlvGFl1Pl1vilvcYtr1lrtl1r....
d +++ + ++y+l++t+ +F++Pl +ilvcY +Il+ ++ ++
58875 187 DLTSPD-----DVLWYTLYLTITTTFFPLPL1LVCYIL1LCYTWemyqq 230

.....kaaktllvvvvvFvlCWlPyfivl1ldtlc.1s
+++ + +++ +++++ k++k++lv+vvvF+l+ +Py++++l++ +++++
58875 231 nkdarccnpsvpkgrvmKLTkMVLVLVVFILSAAPYHVIQLVNLQMeQP 280

iimsstCelervlptallvtlwLayvNsclNpi1Y<-*
++++++L+y++s +NP++Y
58875 281 TL-----AFYVGYYLSICLSYASSSINPFLY 306

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FIG. 10

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Query: 65 KTVP-DIYTCNLAVADL-VHIVGMPFLI-----HQWARGGEWV-FGGPLCTIITS1DTCN 116
+TV + + NLA+ADL V I+ +PF+I + +++ W+ FG LC + + L +
Sbjct: 6 RTVTMNYFLLNLAIADLLVAILTLFFMIVYS1TNFYSQWYFWISFGSCLCKLYSFLYVLS 65 SEQ ID NO:15

Query: 117 QFACSAIMTVMSVDRYFALVQPFRLTRWRTRYKTIRINLGLWAASFILALPVWVYSKVI 175
+A +TV+S+DRY A+ P + R Y TI A ++A+ VWV S +I
Sbjct: 66 MYASIFTLTVISIDRYLAICHPMMSYKRRLHYTTIMTKR---RAVLVIAV-VWVLSILI 120

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FIG. 11

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Query: 270 IQLVNLQMEQPTLAFYVGYYLSICLSYASSSINPFLYILLSGNFQKRLPQIQRATEKEI 329
+QL L + +PTL F Y +I L YA+S +NPF+YI+L F+KRL + A + ++
Sbjct: 1 LQLTQLSISRPTLTFFVYLYNAAISLGYANSCLNPFVYIVLCETFRKRLVLSVKPAAQQQL 60 SEQ ID NO:16

Query: 330 NNMGN 334
+ N
Sbjct: 61 RTVSN 65

```

FIG. 12

Query: 235 RCCNPSVPKQRXXXXXXXXXXXXXXXXXFILSAAPYHVIQLVNLQMEQPTLAFYVGYLSICL 294
 RC K+R F++ APY + +LV L P + + G LS CL
 Sbjct: 95 RCLEEQ--KRRRQRATKKISTFIGTFLVCFAPYVITRLVELFSTAPIDSHW-GV-LSKCL 150 SEQ ID NO:17

Query: 295 SYASSSINPFLYILLSGNFQKRLPQIQRR-TEKEINNMGNTLKSH 339
 +Y+ ++ +PF+Y LL +++ ++ R + I+++G T SH
 Sbjct: 151 AYSKAASDPFVYSLLRHQYRRSCKELNRIFNRRSIHSVGLTGDSH 196

FIG. 13

Query: 26 YQTASVVDTVILPSMIGIICSTGLVGN--LIVFTIIRSRRKTVDPDIYTCNLAVADLVHI 83
 Y+T V T I +++G + + ++ + L+ I+ K + Y L V DL+ +
 Sbjct: 4 YRTY-VASTYISFNVVGFVINAWVLYVVAPLLFAPAIVKPKSIL--FYIFALCVGDLMTM 60 SEQ ID NO:18

Query: 84 VGMPFLIHQWARGGEWVFGGPLCTIITS�DTCNQFACSAIMTVMSVDRYFAL-VQPFRLT 142
 + M L+ + G W F +CT D+ N+F I+ ++S Y + + R
 Sbjct: 61 IAMLLLVIELVFG-TWQFSSMVCTSYLIFDSMNKFMAPMIVFLISRTCYSTVCLDKTRGE 119

Query: 143 RWRT-RYKTIRINLGLWAASFILALPVVYVSKVIKFDGVESCAFDLT 189
 + T +Y I+ + +A IL PV+ YS+V F S A ++T
 Sbjct: 120 KAATLKYAIIQFCIA-FAFVMILLWPVFAYSQVFTTFYMNPNSTAQEV 166

FIG. 14